



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Study on chemical constituents from leaves of camellia

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ABSTRACT

To study the chemical constituents of *Camellia japonica* L. Its chemical constituents were separated by using various modern chromatographic methods, using physicochemical properties combined with the spectral data of compounds. The isolation of 11 compounds, respectively quercetin, I, (-)-epicatechin, II, tellimagrandin II, III, strictinin, IV, 1, 2, 3, 4, 6-penta-O-galloyl- β -D-glucose, V, pedunculagin, VI, heterophylliin A, VII, casuariin, VIII, camelliattannin A, IX, camelliattannin B, X, camelliattannin C, XI. Conclusion: all the compounds were obtained from *Camellia japonica* L.

Keywords: Theaceae; *Camellia japonica*; *Camellia japonica* L

INTRODUCTION

Camellia japonica L is the plant of *Camellia* *Camellia* leaves. Produced in Jiangsu, Zhejiang, Yunnan, Sichuan region in China. It is used For the treatment of epistaxis and hematemesis, folk, intestinal bleeding, dysentery, bleeding hemorrhoids, woman metrorrhagia and chylous hematuria disease^[1]. Tannin were reported abroad *Camellia japonica* L on animal skin cancer and colorectal cancer have obvious inhibition^[2]. So far, the study on the chemical constituents of the little *Camellia japonica* L in China is rare. In order to promote the development of tea plant resources, looking for its active ingredient, the material basis for further research on the efficacy of the constituents, the experiment of the system for the purchase of chemical constituents from Yunnan mountain tea, 11 compounds have been identified as from, respectively quercetin, I, (-)-epicatechin, II, tellimagrandin II, III, strictinin, IV, 1, 2, 3, 4, 6-penta-O-galloyl- β -D-glucose, V, pedunculagin, VI, heterophylliin A, VII, casuariin, VIII, camelliattannin A, IX, camelliattannin B, X, camelliattannin C, XI. All the compounds were obtained from the *Camellia japonica* L.

EXPERIMENTAL SECTION

1 Instruments and materials

Bruker AM600NMR Tester; Shimadzu UV180 Meter UV; JEOL GE XHX100MS; Automatic fraction collector (HL-2) constant flow pump produced in Shanghai City, West Shanghai Machinery Factory; AFQ rotary film evaporator, produced in Tianjin Glass Instrument Factory. Plant samples in 2005 December purchased in medicine market in Yunnan Province, Kunming City, by Professor Zhang Delian identification of traditional Chinese Medicine Department of the Harbin University of Commerce School of medicine, Theaceae *Camellia* *Camellia japonica* L. leaves, sample deposited in Harbin University of Commerce Chinese medicine specimen room.

2 Extraction and separation

Dry *Camellia japonica* L 2 kg, broken with 70% acetone and extracted from filtration, vacuum concentration 40 °C by extraction with ethyl ether, acetic acid. 13g get the ether extract, ethyl acetate extract 45g. By Diaion HP-20 column chromatography separation of ethyl acetate extract, ethanol - water (20% → 40% → 60% → 80%) gradient

elution, by 20% ethanol extract of 23.6g. The ethanol extract 20g, separated by Toyopearl HW-40 column, methanol water (3:7 → 4:6 → 6:4 → 7:3) gradient elution, 4 fractions were obtained (Fr. I .1866mg ; Fr. II .4218mg ; Fr. III. 1700mg ; Fr.IV 1300mg). Fr. I by MCI-gel CHP-20P column chromatography repeatedly isolated compounds I (134mg), compound II (130mg), compound IV (89mg); Fr II by Toyopea HW-40 column chromatography repeatedly isolated compounds VII (69mg), compound VIII (123mg), compound x (110mg), compound Xi (105mg); Fr. III by sephadx LH-20 column chromatography repeatedly isolated compounds IX (100mg), compound (56mg), compound V (47mg); Fr. Toyopearl HW-40, Sephadex LH-20 IV by repeated column chromatography, gets the compound VI (66mg). Normal phase HPLC chromatography using Waters M4 solution, Zorbax SIL column (4.6 mm 150 mm, 5 μ m), the detection wavelength was 280 nm, room temperature, flow rate of 2.5 mL • min⁻¹, mobile phase: cyclohexane and methanol - tetrahydrofuran - formic acid (55:33:11:1) - oxalic acid 450mg • L⁻¹. Shimadzu LC-6A liquid chromatography reversed phase HPLC column, Lichrospher Rp-18 (4 mm 250 mm, 5 μ m), the detection wavelength was 280 nm, temperature 40 °C, mobile phase: phosphate buffer - ethanol - ethyl acetate (85: 10: 5) flow rate of 1.1 mL • min⁻¹.

3 Structure identification

Compound I: light yellow amorphous powder (Acetone) and its molecular formula C₁₅H₁₀O₇ FAB-MS m/z : 325[M+Na]⁺ ; ¹H-NMR(Me₂CO-d₆+D₂O , 300MHz) δ : 6. 23(1H, d, J =2. 1 Hz, H-6) , 6. 49(1H, d, J = 2. 1 Hz , H-8) , 6. 95(1H, d, J = 8. 7 Hz, H-5') , 7. 63(1H, dd, J = 2. 1 , 8. 7 Hz, H-6') , 7. 76(1H, d, J = 2. 1 Hz, H-2') , 13. 02(1H, s, 5-OH) , 10. 12(1H, br s, 7-OH) , 9. 90(3H, br s, 3, 3', 4' -OH) ; ¹³C-NMR (126MHz, Me₂CO-d₆+D₂O) δ : 147. 1(C-2) , 135. 9(C-3) , 176. 9(C-4) , 156. 4(C-5) , 99. 7(C-6) , 165. 1(C-7) , 95. 3(C-8) , 162. 4(C-9) , 105. 2(C-10) , 122. 5(C-1') , 114. 6(C-2') , 146. 2(C-3') , 148. 2(C-4') , 112. 4(C-5') , 119. 3(C-6'). These data are consistent with the literature ^[3], therefore, identified as quercetin.

Compound II: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, its molecular formula is C₁₅H₁₄O₆ FAB-MS m/z: 313[M+Na]⁺ ; ¹H-NMR(Me₂CO-d₆+D₂O , 300MHz) δ : 4. 85(1H, s, br, H-2) , 4. 18(1H, m, H-3) , 2. 83(1H, dd, J = 4. 5 , 16. 5 Hz, H-4) , 2. 71(1H, s, dd, J =3. 0 , 16. 5 , H-4) , 6. 00(1H, s, d, J = 2. 5 Hz, H-6) , 5. 90(1H, s, d, J = 2. 5 Hz, H-8) , 7.03 (1H, s, d, J = 2 , H-2') , 6.77(1H, s, d, J = 8. 0, H-5') , 6. 81(1H, dd, J = 2. 0 , 8. 0, H-6')。 ¹³C-NMR (126MHz, Me₂CO- d₆+D₂O) δ : 79. 2(C-2) , 66. 7(C-3) , 28. 7(C-4) , 99. 5(C-4 a) , 157. 4(C-5) , 95. 9(C-6) , 157. 3(C-7) , 95.3(C-8) , 156. 8(C-8a) , 131. 6(C-1') , 115. 0(C-2') , 145. 2(C-3') , 145. 1(C-4') , 115. 3(C-5') , 119. 0(C-6')。 These data are consistent with the literature reported ^[4], we identified it as (-) epicatechin.

Compound III: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, gelatin reagent positive reaction, its molecular formula is C₄₁H₃₀O₂₆ FAB-MS m/z: 961[M+Na]⁺ ; ¹H-NMR(Me₂CO-d₆+D₂O , 600MHz) δ : 7. 09 , 6. 99 , 6. 96 (2H, s, galloyl) , 6. 20(1H, d, J = 8. 0. Hz, Glu-1) , 5. 58 (1H, dd, J = 8. 0 , 9. 5 Hz, Glu-2) , 5. 83(1H, t, J = 9. 5 Hz, Glu-3) , 5.20 (1H, t, J = 9. 5 Hz, Glu-4) , 4. 54 (1H, dd, J = 6. 0 , 9. 5 Hz, Glu-5) , 5. 36(1H, dd, J = 6. 0 , 13. 0 Hz, Glu-6) , 3. 87 (1H, d, J =13. 0 Hz, Glu-6)。 ¹³C-NMR (126 MHz, Me₂CO- d₆+D₂O) δ : 93. 8(Glu-1) , 71. 8(Glu-2) , 73. 3(Glu-3) , 70. 8(Glu-4) , 73. 1(Glu-5) , 63. 1(Glu-6) , 168. 8 , 168. 2 , 166. 7 , 166. 2 , 166 0(ester carbonyl). These data are consistent with the literature reported by ^[5], so the tellimagrandin II .

Compound IV: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, gelatin reagent positive reaction, its molecular formula is C₂₇H₂₂O₁₈ FAB-MS m/z: 657[M+Na]⁺ ; ¹H-NMR (600 Mz , Me₂CO-d₆+D₂O) δ : 7.15 (2H, s, galloyl) , 6.70 , 6.55 (1H, s, HHDP) , 5.71 (1H, d, J = 8.0 Hz, Glu-1) , 3.67 (1H, dd, J = 8. 0 , 9.0 Hz, Glu-2) , 3.80(1H, dd, J = 9. 0 , 10.0 Hz, Glu-3) , 4.88 (1H, t, J = 10. 0 Hz, Glu-4) , 4.08 (1H, dd, J = 6. 5 , 10. 0 Hz, Glu- 5) , 5. 30 (1H, dd, J = 6.5, 13.5 Hz, Glu-6) , 3.75 (1H, d, J = 13.5 Hz, Glu-6)。 ¹³C-NMR (126 MHz, Me₂CO -d₆+D₂O) δ : 92.0(Glu-1) , 75.8(Glu-2) , 77.2 (Glu-3) , 69.0 (Glu-4) , 73.1 (Glu-5) , 63.0 (Glu- 6) , 166.8 , 166.4 , 166.1 , (ester carbonyl). These data are consistent with the literature reported by ^[6], so the strictinin.

Compound V: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, gelatin reagent positive reaction, its molecular formula is C₄₁H₃₂O₂₆ FAB-MS m/z : 963 (M+Na)⁺ ; ¹H-NMR (600 Mz, Me₂CO-d₆+D₂O) δ : 7. 15 , 7.10 , 7. 05 , 6. 99 , 6. 96 , (2H , s , galloyl×5) , 6. 29 (1H, d, J = 8. 5 Hz , Glu-1) , 5. 67 (1H, dd, J = 8. 5 , 10. 0 Hz, Glu-2) , 6. 00 (1H, t, J =10. 0Hz, Glu-3) , 5. 56(1H, t, J = 10. 0 Hz , Glu-4) , 4. 54 (1H, ddd, J = 2. 0 , 5. 0 , 10. 0 Hz, Glu-5) , 4.58 (1H, dd, J = 2. 0 , 12. 0 Hz, Glu-6) , 4. 31 (1H, dd, J = 5. 6 , 10. 0 Hz, Glu-6) , ¹³C-NMR(126 MHz , Me₂CO-d₆+D₂O) δ : 93. 4(Glu-1) , 74. 9 (Glu-2) , 74. 4 (Glu-3) , 69. 5(Glu-4) , 72. 1 (Glu-5) , 62. 9 (Glu-6) , 166. 9 , 166. 5 , 166.1 , 165. 8 , 165. 4(ester carbonyl)。

These data are consistent with the literature reported by [7], so the 1, 2, 3, 4, 6-penta-O-galloyl- -D-glucose.

Compound VI: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, gelatin reagent positive reaction, its molecular formula is $C_{34}H_{24}O_{22}$ FAB-MS $m/z: 807[M+Na]^+$; 1H -NMR (600 Mz, $Me_2CO-d_6+D_2O$) δ : 6.63, 6.62(1H in total), 6.58, 6.58(1H in total), 6.53, 6.49(1H in total), 6.31, 6.30(1H in total), 5.40(1H, d, $J = 3.9$ Hz, Glc-1 α), 5.01(1H, d, $J = 8.1$ Hz, Glu-1 β), 5.02(1H, dd, $J = 3.9, 10.2$ Hz, Glu-2 α), 4.81(1H, dd, $J = 8.1, 9.0$ Hz, Glu-2 β), 5.41(1H, d, $J = 10.2$ Hz, Glu-3 α), 5.18(1H, dd, $J = 9.0, 10.2$ Hz, Glu-3 β), 5.04(1H, t, $J = 10.2$ Hz, Glu-4 α), 5.03(1H, t, $J = 10.2$ Hz, Glu-4 β), 4.55(1H, m, Glu-5 α), 4.16(1H, m, Glu-5 β), 5.20(1H, dd, $J = 6.9, 13.2$ Hz, Glu-6 α), 5.24(1H, dd, $J = 6.9, 13.2$ Hz, Glu-6 β), 3.74(1H, d, $J = 13.2$ Hz, Glu-6 α), 3.81(1H, d, $J = 13.2$ Hz, Glu-6 β). ^{13}C -NMR (126 MHz, $Me_2CO-d_6+D_2O$) δ : 91.4(Glu-1 α), 95.1(Glu-1 β), 75.4(Glu-2 α), 78.1(Glu-2 β), 75.6(Glu-3 α), 77.4(Glu-3 β), 69.6(Glu-4 α), 69.5(Glu-4 β), 67.1(Glu-5 α), 72.6(Glu-5 β), 63.4(Glu-6 α), 63.5(Glu-6 β). These data are consistent with the literature reported by [6], so the pedunculagin.

Compound VII: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, gelatin reagent positive reaction positive. FAB-MS $m/z: 809(M+Na)^+$; $C_{34}H_{26}O_{26}$ 1H -NMR (600 Mz, $Me_2CO-d_6+D_2O$) δ : 7.24, 7.03(2H, s, galloyl $\times 2$), 6.62, 6.48(1H, s, HHDP), 6.43(1H, d, $J = 4.0$ Hz, Glu-1), 4.22(1H, dd, $J = 4.0, 10.0$ Hz, Glu-2), 5.56(1H, t, $J = 10.0$ Hz, Glu-3), 5.06(1H, t, $J = 10.0$ Hz, Glu-4), 4.57(1H, dd, $J = 6.5, 10.0$ Hz, Glu-5), 5.26(1H, dd, $J = 6.5, 13.5$ Hz, Glu-6), 3.75(1H, d, $J = 13.5$ Hz, Glu-6), ^{13}C -NMR(126 MHz, $Me_2CO-d_6+D_2O$) δ : 92.9(Glu-1), 70.3(Glu-2), 74.3(Glu-3), 70.6(Glu-4), 70.5(Glu-5), 63.3(Glu-6), 165.4, 167.2, 167.7, 168.3(ester carbonyl). These data are consistent with the literature reported by [8], so the heterophylliin A.

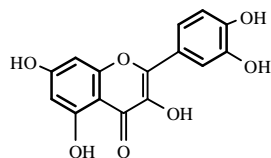
Compound VIII: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, gelatin reagent positive reaction, its molecular formula is $C_{34}H_{24}O_{22}$ FAB-MS $m/z: 807[M+Na]^+$; 1H -NMR (600 Mz, $Me_2CO-d_6+D_2O$) δ : 6.73, 6.50, 6.36, (1H, s, HHDP), 5.54(1H, d, $J = 4.80$ Hz, Glu-1), 4.64(1H, dd, $J = 2.4, 4.8$ Hz, Glu-2), 5.46(1H, d, $J = 2.4$ Hz, Glu-3), 5.07(1H, dd, $J = 2.4, 8.1$ Hz, Glu-4), 4.08(1H, dd, $J = 3.0, 8.1$ Hz, Glu-5), 4.68(1H, dd, $J = 3.0, 12.3$ Hz, Glu-6), 3.81(1H, d, $J = 12.3$ Hz, Glu-6), ^{13}C -NMR (126 MHz, $Me_2CO-d_6+D_2O$) The data in table 1. These data are consistent with the literature reported by [6], so the casuarinin.

Compound IX: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, gelatin reagent positive reaction, its molecular formula is $C_{49}H_{36}O_{27}$ FAB-MS $m/z: 1057[M+H]^+$; 1H -NMR (600 Mz, $Me_2CO-d_6+D_2O$) δ : 6.26, 6.47, 6.65(1H, s, HHDP $\times 2$), 5.03(1H, br, s, Ec-2), 4.24(1H, br, s, Ec-3), 2.85(1H, br, dd, $J = 3.5, 16$ Hz, Ec-4), 2.73(1H, br, d, $J = 16$ Hz, Ec-4), 5.91(1H, s, Ec-6), 7.18(1H, br, s, Ec-2'), 6.93(1H, br, d, $J = 8$ Hz, Ec-5'), 6.83(1H, br, d, $J = 8$ Hz, Ec-6'), 4.71(1H, br, s, Glu-1), 4.93(1H, br, s, Glu-2), 4.99(1H, br, d, $J = 3$ Hz, Glu, H-3), 5.07(1H, br, dd, $J = 3.0, 7.0$ Hz, Glu-4), 3.81(1H, d, $J = 7.0$ Hz, Glu-5), 4.48(1H, br, d, $J = 12.0$ Hz, Glu-6), 3.38(1H, br, d, $J = 12$ Hz, Glu-6). ^{13}C -NMR (126 MHz, $Me_2CO-d_6+D_2O$) The data in table 1. These data are consistent with the literature reported by [9], so the camelliatannin A.

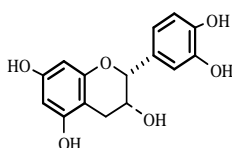
Compound X: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, gelatin reagent positive reaction, its molecular formula is $C_{49}H_{36}O_{27}$ FAB-MS $m/z: 1057[M+H]^+$; 1H -NMR (600 Mz, $Me_2CO-d_6+D_2O$) δ : 6.47, 6.53, 6.80(1H, s, HHDP $\times 2$), 4.78(1H, br, s, Ec-2), 4.15(1H, m, Ec-3), 2.82(1H, br, dd, $J = 3.5, 16.0$ Hz, Ec-4), 2.67(1H, br, d, $J = 16.0$ Hz, Ec-4), 5.98(1H, s, Ec-8), 7.01(1H, d, $J = 1.5$ Hz, Ec-2'), 6.74(1H, d, $J = 8.5$ Hz, Ec-5'), 6.79(1H, dd, $J = 1.5, 8.5$ Hz, Ec-6'), 4.66(1H, d, $J = 1.0$ Hz, Glu-1), 4.82(1H, dd, $J = 1.0, 2.5$ Hz, Glu-2), 5.21(1H, t, $J = 2.5$ Hz, Glu, H-3), 5.27(1H, dd, $J = 2.5, 8.0$ Hz, Glu-4), 4.12(1H, ddd, $J = 1.0, 3.0, 8.0$ Hz, Glu-5), 4.73(1H, dd, $J = 3.0, 12.0$ Hz, Glu-6), 3.83(1H, d, $J = 1.0, 12.0$ Hz, Glu-6). ^{13}C -NMR (126 MHz, $Me_2CO-d_6+D_2O$) The data in table 1. The above data are the same as literature reported [9], which can be used to determine the camelliatannin B.

Compound XI: light brown amorphous powder (Acetone), reaction with ferric chloride reagent blue, gelatin reagent positive reaction, its molecular formula is $C_{49}H_{38}O_{28}$ FAB-MS $m/z: 1075[M+H]^+$; 1H -NMR (600 Mz, $Me_2CO-d_6+D_2O$) δ : 6.53, 6.73, 6.64, 6.64(1H, s, HHDP $\times 2$), 4.78(1H, br, s, Ec-2), 4.17(1H, m, Ec-3), 2.94(1H, dd, $J = 4.5, 17$ Hz, Ec-4), 2.74(1H, dd, $J = 2.5, 17$ Hz, Ec-4), 5.84(1H,

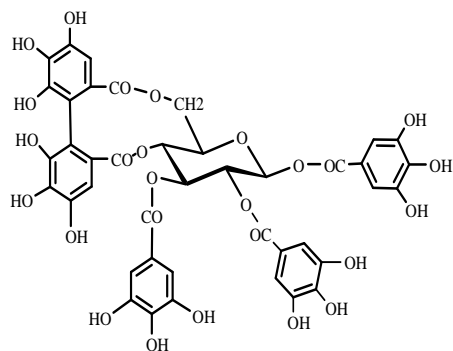
s , Ec-8) , 6.99(1H , d , J = 2.0 Hz , Ec-2') , 6.74(1H , d , J = 8.5 Hz , Ec-5') , 6.78(1H , dd , J = 2.0 , 8.5 Hz , Ec-6') , 5.75(1H , d , J = 2.0 Hz , Glu-1) , 5.42(1H , dd , J = 2.0 , 9.0 Hz , Glu-2) , 5.89(1H , dd , J = 2.0 , 9.0 Hz , Glu , H-3) , 5.53(1H , dd , J = 2.0 , 8.0 Hz , Glu-4) , 4.32(1H , br , d , J = 8.0 Hz , Glu -5) , 4.60(1H , dd , J = 2.5 , 12.5 Hz , Glu-6) , 3.98(1H , d , J = 12.5 Hz , Glu-6) . $^{13}\text{C-NMR}$ (126 MHz, $\text{Me}_2\text{CO-d}_6 + \text{D}_2\text{O}$) The data in table 1. These data are the same as the consistent in the literature report^[10], which can be used to identify the camelliatannin C.



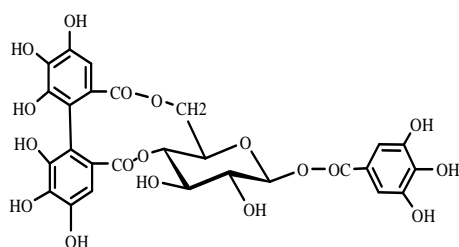
quercetin (I)



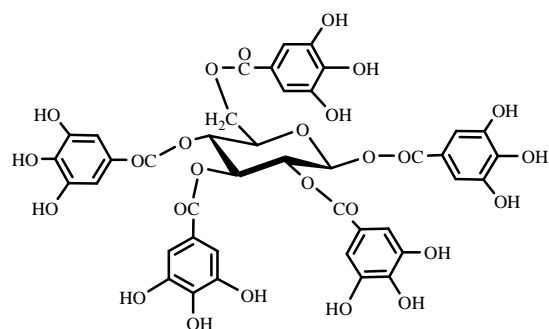
(-)epicatechin(II)

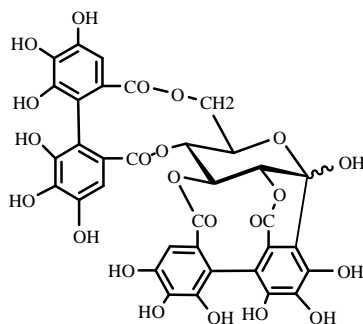


tellimagrandin II (III)



strictinin(IV)

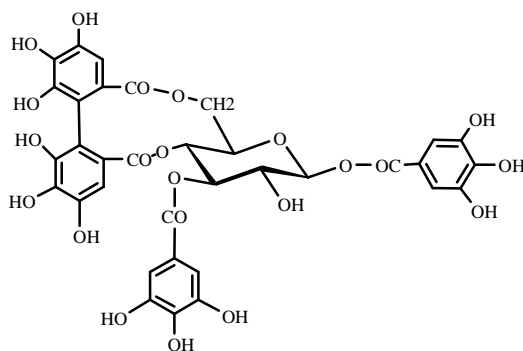
1,2,3,4,6-penta-O-galloyl- β -D-glucose(V)



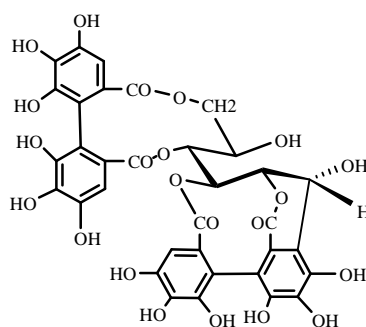
pedunculagin(VI)

Table 1 ¹³C-NMR Data of compound VIII-XI

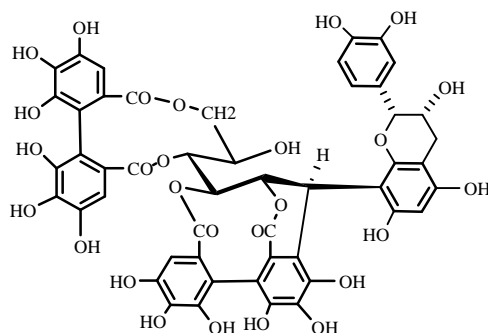
	碳位	VIII	IX	X	XI				
Glucose	C-1	67.7	38.2	38.1	68.3				
	C-2	76.9	80.3	81.5	78.6				
	C-3	70.7	76.5	76.4	76.5				
	C-4	77.1	75.8	75.9	72.2				
	C-5	68.4	69.2	68.6	69.1				
	C-6	68.1	67.1	67.9	67.9				
HHDP	C-1, 1'	115.1	115.9	115.4	115.6	115.4	115.8	114.9	116.8
		116.3	116.6	115.7	116.9	115.9	117.0	113.9	114.1
	C-2, 2'	120.5	125.5	122.2	124.2	122.4	124.0	124.9	127.2
		127.4	127.6	125.9	127.2	125.9	127.4	127.5	127.6
	C-3, 3'	105.1	106.9	105.2	107.4	105.8	107.3	106.7	109.1
		108.3	115.9	108.6	128.0	108.9	128.1	107.5	107.9
	C-4, 4'	145.0	145.1	145.0	145.2	145.1	145.2	145.1	145.1
		145.7	146.2	145.2	145.4	145.2	145.7	145.1	145.2
	C-5, 5'	134.8	135.6	134.9	135.8	135.2	155.9	135.4	136.8
		136.5	138.4	136.3	137.5	136.5	137.6	135.8	136.0
C-6, 6'	143.6	143.8	142.7	143.3	143.1	143.2	143.8	144.1	
	144.3	144.5	144.2	144.2	144.3	144.4	144.3	143.3	
C-7, 7'	164.7	168.6	167.2	167.8	167.9	168.7	169.7	167.3	
	169.3	170.2	169.1	170.4	169.6	170.6	169.4	168.8	
Epicatechin	C-2		79.4		70.0		79.1		
	C-3		66.5		66.6		66.6		
	C-4		29.1		29.3		28.9		
	C-4		99.6		100.1		99.1		
	C-5		156.4		155.6		157.1		
	C-6		96.6		107.2		103.8		
	C-7		155.9		155.6		155.7		
	C-8		105.2		96.5		94.9		
	C-8		153.9		155.2		153.3		
	C-1'		132.0		131.9		131.9		
	C-2'		115.0		115.0		115.1		
	C-3'		145.0		145.2		145.1		
C-4'		145.1		144.3		144.7			
C-5'		115.7		115.4		115.3			
C-6'		119.4		119.2		119.5			



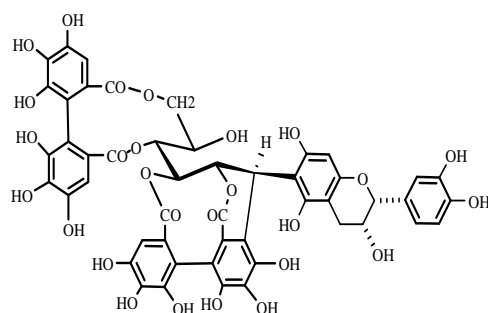
heterophyllin A (VII)



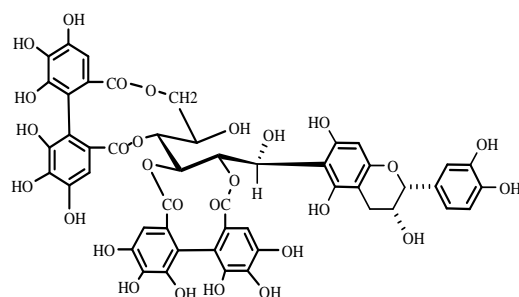
casuariin (VIII)



camelliatannin A (IX)



camelliatannin B (X)



camelliatannin C (XI)

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