·化学成分·

Tannins from *Corylus heterophylla* (Ⅲ)

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Abstract: Objective To study tannins of *Corylus heterophylla* from Heilongjiang Province. **Methods** The constituents were separated and purified repeatedly by macroporons resin chromatography methods and their structures were identified by ^{1}H-NMR, ^{13}C-NMR, FAB-MS, and ^{1}H-^{1}HCOSY spectra, and chemical methods as well. **Results** Three tannins were obtained from *C. heterophylla*. They were heterophylliin D (Ⅰ), roxbin A (Ⅱ), and rugosin F (Ⅲ). **Conclusion** Heterophylliin D (Ⅰ) is isolated from the extract of *C. heterophylla* leaves as a new dimmer tannin firstly.

Key words: Betulaceae; *Corlus* L.; *Corylus heterophylla* Fisch. ex Bess.; tannins

平榛鞣质成分研究(Ⅲ)

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摘 要:目的 研究黑龙江省榛(平榛)*Corylus heterophylla* 的鞣质成分。**方法** 利用大孔树脂柱色谱分离纯化技术反复分离纯化,通过^{1}H-NMR、^{13}C-NMR、FAB-MS、^{1}H-^{1}HCOSY等光谱手段及化学方法对得到的单体成分进行结构鉴定。**结果** 从该植物叶中分离得到3个鞣质成分:榛叶鞣质D(Ⅰ)、刺梨素A(Ⅱ)、玫瑰素F(Ⅲ)。**结论** 榛叶鞣质D(Ⅰ)为一新的二聚体鞣质成分。

关键词:桦木科;榛属;平榛;鞣质

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1 Intruduction

Corylus heterophylla Fisch. ex Bess. is widespread in the northern part of China. Its fruits have been used as folk medicines for the treatment of stomach upset and bowel disorders. The medicinal value of its leaves has not been investigated previously. In the previous report, we isolated and characterized four hydrolyzable tannins from this plant. As a further survey of the distribution of tannins from *C. heterophylla*, we obtained another new tannin compound named as heterophylliin D (Ⅰ)[1] and two known compounds: roxbin A (Ⅱ), rugosin F (Ⅲ).

2 Experiment

2.1 Plant and apparatus: Leaves of *C. heterophylla* were collected in Harbin, Heilongjiang Province, China, in July, 2003 and identified by Prof. Zhang De-Lian, Harbin Commerce University.

^{1}H-NMR (500 MHz) and ^{13}C-NMR (126 MHz) were measured in Me_2CO-d_6-D_2O. Optical rotations were measured on a JASCO DIP-4 Digital Polarimeter at 25 ℃. FAB-MS was measured with 3-nitrobenzyl alcohol matrix. RP-HPLC was performed on a LiChrospher RP-18 column developing with 0.01 mol/L H_3PO_4-0.01 mol/L K_2HPO_4-EtOH-EtOAc (9∶9∶4∶2). Column chromatography (CC) was performed with Diaion HP-20, Sephadex LH-20 (Mitsubishi Kasei Industry Co., Ltd., Japan).

2.2 Extraction and isolation: The concentrated solution of *C. heterophylla* and the aqueous Me_2CO homogenate of the leaves (3.0 kg) was fractionated by CC over Diaion HP-20 (55 cm×14 cm) with H_2O and aqueous MeOH in a stepwise gradient-

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mode: H_2O, 20% MeOH, 30% MeOH, 40% MeOH, 50% MeOH, MeOH, and MeOH-H_2O-acetone (6 ∶ 2 ∶ 2). The eluate (20 g) of the MeOH-H_2O-acetone (6 ∶ 2 ∶ 2) (48 g) was further submitted to a combination of CC over Sephadex LH-20 (51 cm × 5 cm) and Ⅰ (41 mg) was got. The 50% MeOH eluate was chromatographed repeatedly over Sephadex LH-20 to give roxbin A (Ⅱ) (65 mg), rugosin F (Ⅲ) (53 mg).

3 Identification

Compound Ⅰ: Off-white amorphous powder, $[\alpha]_D^{26}$ + 77° (*c* = 1.0, MeOH). FAB-MS *m/z*: 1 893 $(M + Na)^-$. UV λ_{max}^{MeOH} (log ε): 215 nm (5.27), 255 nm (4.88). CD (MeOH) $[\theta]$ (nm): +29.3 × 10^4 (236), −10.3 × 10^4 (261), 3.1 × 10^4 (282). ^{1}H-NMR (acetone-d_6-D_2O) δ: 7.13 [2H, s, galloyl (G)], 7.16, 6.64, 6.52, 6.49, 6.43, 6.39, 6.38, 6.34, 6.20 [each 1H, s, hexahydroxydiphenoyl (HHDP) and valoneoyl (Val)], 6.08, 6.13 (each d, *J* = 8.5 Hz, glu H-1, 1′), 5.35, 5.38 (each dd, *J* = 9.5, 10 Hz, glu H-3, 3′), 5.26, 5.20 (each dd, *J* = 7, 13 Hz, glu H-6, 6′), 5.13, 5.17 (each dd, *J* = 8.5, 9.5 Hz, glu H-2, 2′), 5.07, 5.05 (each t, *J* = 10 Hz, glu H-4, 4′), 4.40 (m, glu H-5, 5′), 3.74, 3.79 (each d, *J* = 13 Hz, glu H-6, 6′). ^{13}C-NMR (acetone-d_6-D_2O) δ: 92.0, 91.9 (glu C-1, 1′), 75.8, 75.7 (glu C-2, 2′), 77.1 (glu C-3, 3′), 69.0 (glu C-4, 4′), 73.4, 73.3 (glu C-5, 5′), 63.0, 62.9 (glu C-6, 6′), 104.8, 107.1 (2C), 107.3, 107.4, 107.5, 107.7, 108.1, 109.7 (HHDP C-3, 3′, Val C-3, 3′, 6″), 110.1 (2C) (G C-2, 6), 113.3, 114.3, 114.6, 114.8, 114.9, 115.4, 115.7, 116.0, 117.1 (HHDP C-1, 1′, Val C-1, 1′, 1″), 119.6 (G C-1), 125.2, 125.5, 125.6, 125.8, 125.9, 126.0, 126.1, 126.2 (HHDP C-2, 2′, Val C-2, 2′), 136.1, 136.3, 136.4, 136.45 (2C), 136.54 (2C), 136.9, 137.5 (HHDP C-5, 5′, Val C-5, 5′, 2″), 139.9 (G C-4), 140.5, 141.2 (Val C-3″, 4″), 145.0 (2C) (G C-3, 5), 143.4, 144.1, 144.2, 144.3 (2C), 144.4 (2C), 144.6, 144.9, 145.1, 145.2, 146.1, 146.8 (HHDP C-4, 4′, 6, 6′ Val C-4, 4′, 6, 6′, 5″), 169.2, 169.1, 168.7, 168.1, 167.9 (2C), 167.6, 165.0, 163.0 (ester carbonyl).

Degalloylation of Ⅰ: A mixture of Ⅰ (30 mg) and tannase in water (3 mL) was incubated at 37 ℃ for 5 h. The reaction mixture, after adding dilute HCl, was concentrated and the residue was subjected to CC over Sephadex LH-20 with H_2O, 10% MeOH, 30% MeOH, and 40% MeOH in a stepwise-mode to give gallic acid (10% MeOH eluate) and roxbin A (Ⅱ) (40% MeOH eluate), which was identified by ^{1}H-NMR spectral compatrison with an authentic sample.

Compound Ⅱ: A light brown amorphous powder, $[\alpha]_D^{26}$ + 46.5° (*c* = 1.0, MeOH). ^{1}H-NMR (acetone-d_6-D_2O) δ: 7.13 (1H, s, G), 6.63, 6.52, 6.48, 6.45, 6.39, 6.36, 6.34, 6.20 (HHDP and VAL), 6.06, 6.15 (each d, *J* = 8.5 Hz, glu H-1, 1′), 5.34, 5.37 (each dd, *J* = 9.5, 10 Hz, glu H-3, 3′), 5.26, 5.20 (each dd, *J* = 7, 13 Hz, glu H-6, 6′), 5.13, 5.17 (each dd, *J* = 8.5, 9.5 Hz, glu H-2, 2′), 5.07, 5.05 (each t, *J* = 10 Hz, glu H-4, 4′), 4.40 (m, glu H-5, 5′), 3.73, 3.77 (each d, *J* = 13 Hz, H-6, 6′). ^{13}C-NMR (acetone-d_6-D_2O) δ: 94.2 (glu C-1), 72.2 (glu C-2), 73.6 (glu C-3), 71.1 (glu C-4), 73.6 (glu C-5), 63.5 (glu C-6).

Compound Ⅲ: A light brown amorphous powder, $[\alpha]_D^{26}$ + 88° (*c* = 1.0, MeOH). ^{1}H-NMR (acetone-d_6-D_2O) δ: 7.14 (2H, s, G), 7.12 (2H, s, G), 7.12 (2H, s, G), 7.15 (1H, s, G), 6.62, 6.53, 6.46, 6.44, 6.39, 6.36, 6.34, 6.20 (HHDP and Val), 6.06, 6.15 (each d, *J* = 8.5 Hz, glu H-1, 1′), 5.34, 5.37 (each dd, *J* = 9.5, 10 Hz, glu H-3, 3′), 5.26, 5.20 (each dd, *J* = 7, 13 Hz, glu H-6, 6′), 5.13, 5.17 (each dd, *J* = 8.5, 9.5 Hz, glu H-2, 2′), 5.06, 5.04 (each t, *J* = 10 Hz, glu H-4, 4′), 4.40 (m, glu H-5, 5′), 3.73, 3.76 (each d, *J* = 13 Hz, glu H-6, 6′). ^{13}C-NMR (acetone-d_6-D_2O) δ: 94.2 (glu C-1), 72.2 (glu C-2), 73.6 (glu C-3), 71.1 (glu C-4), 73.7 (glu C-5), 63.4 (glu C-6).

4 Results and discussion

Compound Ⅰ: Fast atom bombardment mass spectrum (FAB-MS) showed a pseudomolecular

ion peak at m/z: 1 893 $[M+Na]^+$. A 2H singlet and nine 1H singlets in the aromatic region of the ^{1}H-NMR spectrum are attributable to a G group, a Val group, and three HHDP groups. The ^{1}H-NMR spectrum of I showed the sugar was fully acylated (seen in Table 1). The ^{13}C-NMR spectrum showed 12 glucose carbon signals exhibiting as six pair-like signals (1 : 1), indicating analogy of the substitutents on each glucose core. Ten ester carbonyl carbon resonances in the ^{13}C-NMR spectrum were consistent with the presence of these groups (seen in Table 1). The absolute configuretion of each HHDP and VAL group in I was determined to be (*S*)-series by the strong positive Cotton effect at 236 nm in the circular dichroism (CD) spectrum[2]. *Based on these spectral data, compound* I was deduced to be a dimer formed byC-O oxidative coupling between two moles of casuarictin (Ⅳ). The structure of I was confirmed by its chemical conversion into a known dimmer roxbin A (Ⅱ)[3] and by enzymatic degalloylation. The structure of I is thus assigned to heterophylliin D (Fig. 1).

Table 1 ^{1}H-NMR and ^{13}C-NMR Chemical shifts of I and Ⅳ (500 MHz, acetone-d_6)

Protons	I		Ⅳ	
	δ_H	δ_C	δ_H	δ_C
C-1	6.08 (d, *J*= 9)	92.0	6.22 (d, *J*= 9)	92.4
C-1′	6.13 (d, *J*= 9)	91.8		
C-2	5.13 (t, *J*= 9)	75.8	5.18 (t, *J*= 9)	76.0
C-2′	5.13 (t, *J*= 9)	75.75		
C-3	5.40 (dd, *J*= 9, 10)	77.1	5.45 (dd, *J*= 9, 10)	77.3
C-3′	5.40 (dd, *J*= 9, 10)	77.0		
C-4	5.07 (t, *J*= 10)	69.0	5.17 (t, *J*= 10)	69.3
C-4′	5.05 (t, *J*= 10)	69.0		
C-5	4.40 (dd, *J*= 7, 10)	73.3	4.50 (dd, *J*= 7, 10)	73.3
C-5′	4.40 (dd, *J*= 7, 10)	73.3		
C-6	5.26 (dd, *J*= 7, 13)	63.0	5.30 (dd, *J*= 7, 13)	63.1
C-6′	5.21 (dd, *J*= 7, 13)	62.9		
C-6	3.80 (d, *J*= 13)		3.88 (d, *J*= 13)	
C-6′	3.75 (d, *J*= 13)			
G-H	7.13 (2H, s)		7.18 (2H, s)	
HHDP-H	6.64, 6.49, 6.43, 6.39, 6.38, 6.34 (each 1H, s)		6.65, 6.55, 6.47, 6.38, 6.38, 6.34 (each 1H, s)	
Val-H	7.16, 6.52, 6.20 (each 1H, s)			

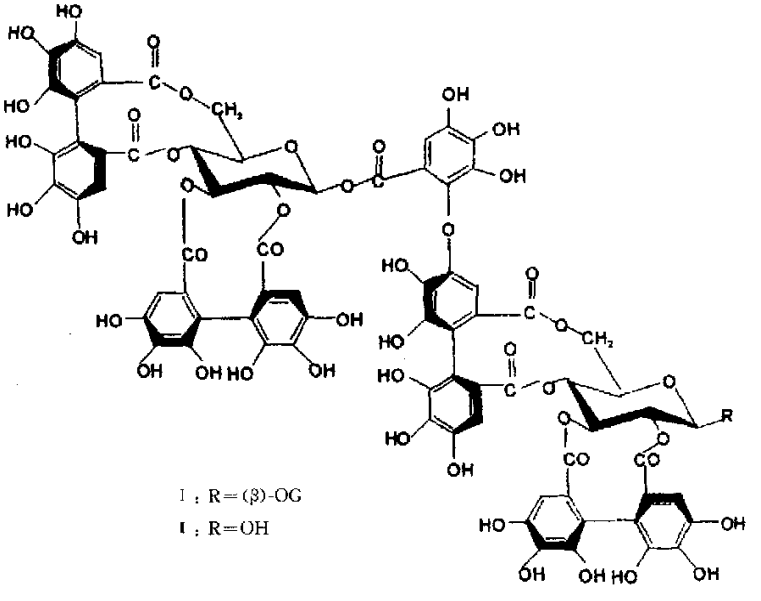

Fig. 1 Sturcture of compounds I and Ⅱ

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