Studies on the Chemical Constituents of Turpinia arguta Seem

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Abstract. From the *Turpinia arguta* Seem, twelve compounds were isolated and identified as corosolic acidacid-28-O-β-D-glucopyranosyl ester(1), pomolic acid(2), ursolic acid(3), quercetin (4), rhoifolin (5), apigenin-7-O-rutinoside(6), quercetin-3- O-a-L-ara- binopyranoside(7), p-hydroxy cinnamic acid(8), caffeic acid(9), gallic acid, (10) 3,4-dihydroxybenzonic acid(11), p-hydroxybenzoic acid(12). Their structures were determined by ^{1}H-NMR , ^{13}C-NMR spectral data.

Introduction

Turpinia arguta Seem is distributed in the southern part of China. The leaves of Turpiniae arguta Seem has been used as an antibacterial, anti-inflammatory and analgesia agent in China for thousands.Flavonoids, megastigmanes and triterpenoids have been identified as constituents of the plant[1,2]. In the present paper, twelve compounds were isolated and identified as corosolic acidacid-28-O-β-D-glucopyranosyl ester(1), pomolic acid(2), ursolic acid(3), quercetin (4), rhoifolin (5), apigenin-7-O-rutinoside(6), quercetin-3- O-a-L-ara- binopyranoside(7), p-hydroxy cinnamic acid(8), caffeic acid(9), gallic acid, (10) 3, 4-dihydroxybenzonic acid(11), p-hydroxybenzoic acid(12) by means of ^{1}H-NMR , ^{13}C-NMR spectral data.

Experimental

General experimental procedures All melting points were determined on a X-4A micro-melting point apparatus and are uncorrected. IR spectra were recorded with an American Nicolet IMPACT-400 Fourier Transform Infrared Spectrometer. ^{1}H-NMR, ^{13}C-NMR, HSQC and HMBC were recorded on an American Varian company INOVA-400 and SYSTEM-600 FT NMR spectrometer. Silica gel (200–300 mesh; Qingdao Marine Chemical Plant) was used for column chromatography.

Plant material *Turpinia arguta Seem* were collected at Ganzhou, Jiangxi Province, China in September of 2013 and authenticated by Professor Q. Lui, Jiangxi University of Traditional Chinese Medicine. A voucher specimen (13-09-18) of the plant is deposited at the Herbarium of Jiangxi University of Traditional Chinese Medicine.

Extraction and isolation The leaves of *Turpiniae arguta* Seem was reduced to a coarse powder and refluxed with 95% ethanol three times, After evaporation of ethanol in vacuo, the aqueous residue was diluted with water and was extracted with chloroform, ethyl acetate and n-butanol saturated with water to give the respective extracts after solvent removal. The ethyl acetate-solution portion(58g) was subjected to column chromatography on silica gel using a gradient mixture of

chloroform-methanol (from 95:5 to 50:50) as eluting solvent to afford compound 2, 3, 4, 11, 12. The n-butanol-solution portion was evaporated, and the part extract(90g) was fractionated by silica gel column chromatography, eluted with $CHCl_3$–MeOH–H_2O(6:4:1) to give five fractions (Fractions I–V). Fraction Ⅲwas subjected to a silica-gel column eluted with $CHCl_3$–MeOH–H_2O (7:3:1) and preparative HPLC [H_2O–MeOH (60:40), 5ml/min, monitored at 285 nm] to give compound 5~10. Fraction V was subjected to preparative HPLC [H_2O–CH_3CN (70:30), 5ml/min, monitored at 210 nm] to afford compound 1.

Results and discussion

Compound 1 was white powder, mp242～244℃. ^{1}H-NMR (C_5D_5N, 400 MHz)*δ*: 0.96 (3H,d, J=6Hz), 0.99 (3H, d, J=6.2 Hz), 1.01 (3H, s), 1.05(3H, s), 1.07 (3H, s), 1.23 (3H, s), 1.28 (3H, s) , 3.36 (1H, d, J=9Hz), 4.15 (1H,td, J=11,4Hz), 5.44 (1H, t, J=3Hz). ^{13}C-NMR(C_5D_5N, 100 MHz) δ：48.1(C-1), 68.8(C-2), 83.1(C-3), 39.3(C-4), 55.2(C-5), 18.7(C-6), 33.1(C-7), 40.4(C-8), 48.2(C-9), 38.2(C-10), 23.8(C-11), 125.8(C-12), 139.4(C-13), 42.6(C-14), 28.1(C-15), 24.6(C-16), 48.1(C-17), 53.2(C-18), 39.5(C-19), 39.3(C-20), 31.8(C-21), 37.6(C-22), 29.6(C-23), 17.0(C-24), 17.2(C-25), 17.6(C-26), 23.6(C-27), 177.6(C-28), 17.2(C-29), 21.5 (C-30), 95.2(C-1’), 74.1(C-2’), 79.3(C-3’), 71.2(C-4’), 78.2(C-5’),62.3 (C-6’). On the basis of relevant reference [3] and its spectral data, its structure was elucidated to be corosolic acidacid-28-*O*-*β*-D-glucopyranosyl ester.

Compound 2 was white powder, mp：298-300℃. ^{1}H-NMR（$CDCl_3$, 400MHz）δ: 0.88（3H, s）, 1.05（3H,d , J=6 Hz）, 1.11（3H,s）,1.16（3H,s）,1.18（3H,s）,1.37（3H,s）,1.69（3H,s）, 3.25 (1H, dd, J=11,5 Hz), 5.59 (1H, t, *J*=3 Hz). ^{13}C-NMR($CDCl_3$, 100MHz) δ：39.7(C-1), 26.4(C-2), 78.0(C-3), 39.3(C-4), 56.3(C-5), 18.6(C-6), 33.3(C-7), 40.5(C-8), 47.4(C-9), 37.2(C-10), 23.9(C - 11), 128.2(C-12), 139.1(C-13), 42.3(C-14), 29.4(C-15), 25.6(C-16), 48.8(C-17), 53.3(C-18), 72.5 (C-19), 42.2(C-20), 26.8(C-21), 37.6(C-22), 27.9(C-23), 17.3(C-24), 16.0(C-25), 17.0(C-26), 24.5 (C-27), 180.9(C-28), 27.3(C-29), 16.3 (C-30). On the basis of relevant reference [4] and its spectral data, its structure was elucidated to be pomolic acid.

Compound 3 was white powder, m p270～271℃, ^{1}H NMR(C_5D_5N, 400MHz) *δ*: 0.91 (3H, s), 0.98 (3H, d, J=6.0 Hz), 1.02 (3H, d, *J*=6.4 Hz), 1.03 (3H, s), 1.07 (3H,s), 1.27 (3H, s), 1.29 (3H, s),3.42 (1H, dd, J=10,7Hz),5.54 (1H, t, J=3.2 Hz). ^{13}C-NMR(C_5D_5N, 100MHz) δ：39.1(C-1), 28.1(C-2), 78.1(C-3), 39.2(C-4), 55.3(C-5), 18.3(C-6), 33.3(C-7), 40.4(C-8), 48.1(C-9), 37.0(C-10), 23.7(C-11), 126.2(C-12), 139.3(C-13), 42.6(C-14), 28.8(C-15), 24.9(C-16), 48.0(C-17), 53.2(C-18), 39.5(C-19), 39.2(C-20), 31.8(C-21), 37.5(C-22), 27.9(C-23), 15.3(C-24), 16.0(C-25), 17.7(C-26), 23.5(C-27), 179.9(C-28), 17.3(C-29), 21.3 (C-30). On the basis of relevant reference [5] and its spectral data, its structure was elucidated to be ursolic acid.

Compound 4 was obtained as a yellow needle crystals, ,mp 311~313℃, ^{1}H NMR(DMSO-d_6, 400 MHz)δ :12.47 (1H, s), 7.66(1H, d, *J* = 2.2 Hz), 7.52(1H, d, *J* = 7.8 Hz), 6.86 (1H, d, *J* = 8.4 Hz), 6.38 (1H, d, J = 2.0 Hz), 6.16(1H, d, *J* = 2.0 Hz). ^{13}C NMR(DMSO-d_6, 100 MHz)δ: 175.7 (C-4), 164.4 (C-7), 160.6 (C-9), 156.1 (C-5), 147.7 (C-4'), 146.6 (C-2), 145.0 (C-3'), 135.6 (C-3), 121.9 (C-1'), 119.9 (C-6'), 115.5 (C-5'), 114.9 (C-2'), 102.7 (C-10), 98.3 (C-6), 93.3 (C-8). On the basis of spectral data[6], its structure was elucidated to be quercetin.

Compound 5 were pale yellow granules, soluble in methanol, mp248-250℃, ^{1}H NMR (DMSO -d_6, 400 MHz) δ: 12.96 (1H, s, OH), 10.43 (1H, s, OH) , 7.94 (2H, d, J = 8.8 Hz, H-2 ', 6'), 6.94 (2H, d, J = 8.8 Hz, H-3', 5'), 6.87 (1H, s, H-3), 6.79 (1H, d, J = 2.1 Hz, H-8), 6.38 (1H, d, J = 2.1 Hz, H-6). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 182.45 (C-4), 164.74 (C-2), 161.86 (C-7), 161.58 (C-5), 157.45 (C-9), 129.05 (C-2 ', 6), 121.46 (C-1'), 116.50 (C-3', 5'), 105.89 (C-10), 103.71 (C-3), 100.93(C-1''), 98.27 (C-6), 98.32 (C-1'''), 94.98 (C-8), 77.68 (C-2''), 77.48 (C-3''), 76.72 (C- 5''), 72.33 (C-4''), 70.94 (C-4'''), 70.86 (C-3'''), 70.11 (C-2 '''), 68.81 (C-5'''), 60.93 (C-6''), 18.54 (C-6'''). The above data is consistent with the literatures, this compound is identified as rhoifolin.

Compound 6 was obtained as an amorphous solid.^{1}H- NMR(DMSO-d_6,600MHz) δ: 7.92(2H, d, *J*=9.0Hz, H-2’, H-6’), 6.92（2H, d, *J*=9.0Hz, H-3’, H-5’）, 6.83 (1H,s), 6.76（1H,d,*J*=2.4Hz,8-H）, 6.44(1H,d,*J*=2.4 Hz ,6-H). ^{13}C-NMR(150MHz,DMSO-d_6) δ: 181.9（C-4）,162.8（C-5）,161.1（C-7）,156.8（C-9）,128.6（C-2′,C-6′）, 116.2（C-3′,C-5′）,105.3（C-10）, 100.5（C-1''）, 99.8（C-1'''）, 99.4（C-6）, 94.7（C-8）, 76.2（C-5''）, 75.5（C-3′）, 73.0（C-4'''）, 72.0（2''）, 70.7（C-4''）, 70.3（C-3'''）, 69.5（C-2'''）, 68.2（C-5'''）, 66.0（C-6''）, 17.7（C-6'''）. On the basis of relevant reference [7] and its spectral data, its structure was elucidated to be apigenin- 7- O-rutinoside.

Compound 7 was obtained as a yellow powder. ^{1}H-NMR(DMSO-d_6,600MHz)δ: 7.64(1H,dd,*J*= 9,2 Hz, H-6’), 7.50(1H,d,*J*= 1.8 Hz, H-2’), 6.83(1H,d,*J*= 8.4 Hz, H-5’), 6.38(1H, d, *J*=1.8Hz, H-8), 6.18(1H,d,*J*=1.2Hz, H-6), 5.26（1H,d,*J*=5.4Hz, H-1''）. ^{13}C-NMR(DMSO-d_6,125MHz) δ: 177.4（C-4）, 164.6（C-7）, 161.1（C-5）, 156.3（C-9）, 156.1（C-2）, 148.6（C-4’）, 145.0（C-3’）, 133.6（C-3）, 122.0（C-6′）, 120.8（C-1′）, 115.7（C-5′）, 115.3（C-2′）, 103.7（C-10）, 101.4（C-1”）, 98.7（C-6）, 93.5（C-8）, 71.6（C-2”）, 70.7（C-3”）, 60.0（C-4”）, 64.2（C-5”）On the basis of spectral data[8], its structure was elucidated to be quercetin-3-O-a-L-ara-binopyranoside.

Compound 8 was white powder, soluble in methanol, mp217-219 ℃ . ^{1}H-NMR (DMSO-d_6,400MHz) δ: 12.15 (1H, s, OH), 9.95 (1H, s, OH), 6.28 (1H, d, J = 16Hz, H-3), 7.49 (1H, d, J = 16Hz, H-2), 6.78 (2H, J = 8.8 Hz, H-6,8), 7.50 (2H, d, J = 8.8 Hz, H-5,9). ^{13}C-NMR (DMSO-d_6,100MHz) δ: 168.40 (C-1), 160.05 (C-7), 144.63 (C-3), 130.55 (C-5,9), 125.73 (C-4), 116.20 (C-6,8), 115.79 (C-2). The above data is consistent with the literature, so it is identified as p-hydroxy cinnamic acid.

Compound 9 was obtained as a yellow powder, mp：201-203℃, ^{1}H-NMR（CD_3OD,600MHz）δ: 6.23(d, *J*＝15 Hz), 7.53(d, *J*＝15 Hz), 6.73(d, *J*＝8.4Hz), 6.93(dd, *J*＝7.8 Hz,1.8Hz), 7.03(d, *J*＝1.8 Hz).^{13}C-NMR（CD_3OD,150MHz）δ :171.6(COOH), 115.3（C-6）,116.1（C-5）,117.0（C-2）,122.9（C-8）,128.1（C-1）,146.9（C-3）,147.0（C-8）,149.5（C-4）, 171.6（C-9）. On the basis of relevant reference [9] and its spectral data, its structure was elucidated to be caffeic acid.

Compound 10 was yellow needle crystal, soluble in methanol, mp201-203℃, ^{1}H-NMR (DMSO-d_6,400MHz) δ: 12.22 (1H, s, OH), 9.18 (2H, s, OH), 8.92 (1H, s, OH), 6.92 (2H, s, H-2,6), ^{13}C-NMR (DMSO-d_6,100MHz) δ: 167.91 (COOH), 145.86 (C-3,5), 138.44 (C-4), 120.89 (C-1), 109.17 (C-2,6). The above data is consistent with the literature, it is identified as gallic acid.

Compound 11 was obtained as colorless needles, mp：210~212℃. UVλ_{max}(MeOH)：206nm 254nm. IR（KBr）cm^{-1}：3207, 1676, 1601, 1529, 1298, 1466, 1419, 1381, 1130, 1095, 941, 766, 638, 577. EI-MS m/z (%)：154(M^+,85), 137(100), 119(3), 110(31), 97(5), 91(6), 81(22), 69(3), 63(32), 53(34), 44(35), 41(6). ^{1}H-NMR (CD_3OD, 400MHz)δ： 7.47(1H, s, H-2) 7.45(1H, d, H-6), 6. 82(1H, s, H-5) .^{13}C-NMR (CD_3OD, 100MHz)δ： 170.2, 151.5(C-4), 146.0(C-3), 123.9(C-1), 123.1(C-6), 117.7(C-2), 115.7(C-5). On the basis of spectral data, its structure was elucidated to be 3, 4-dihydroxybenzonic acid.

Compound 12 was white powder. ^{1}H-NMR（CD_3OD,400MHz）δ: 7.86(2H, d, J＝8.4 Hz), 6.82(2H, d, J＝8.4 Hz). ^{13}C-NMR（CD_3OD,100MHz）δ: 163.4（C-1）,132.7（C-3）,116.2（C-2）,123.1（C-4）. On the basis of spectral data[10], its structure was elucidated to be p-hydroxybenzoic acid.

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