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Studies on Chemical Constituents of Twigs of *Trichosanthes kirilowii* Maxim

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In the present study, 12 compounds were isolated from ethyl acetate extract of the twigs of *Trichosanthes kirilowii* Maxim.. Their structures were determined by UV, IR, EI-MS, ESI-MS, 1D and 2D NMR techniques. These compounds were identified as 4-[formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]-butanoic acid (**1**), 3-hydroxy-1-(4-hydroxyphenyl)-1-propanone (**2**), *p*-hydroxybenzoic acid (**3**), 4-(2-formyl-5-hydroxymethylpyrrol-1-yl)-butyric acid (**4**), 2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-propane-1,3-diol (**5**), 2-[4-(3-hydroxy-1-propenyl)-2-methoxyphenoxy]-1,3-propanediol (**6**), (-)-methyl dihydrophaseate (**7**), 4'-dihydrophaseic acid (**8**), 4-hydroxy-3-methyloxybenzoic acid (**9**), quercetin-3-O-rutinoside (**10**), salicylic acid (**11**), 2-methoxy-2-(4'-hydroxyphenyl)-ethanol (**12**). All of the compounds were isolated from the twigs of *Trichosanthes kirilowii* Maxim. for the first time. Compounds **1** to **9**, **11** and **12** were firstly reported from the genus of *Trichosanthes.*

Keywords: Twigs of *Trichosanthes kirilowii* Maxim., Chemical constituents.

INTRODUCTION

Fructus Trichosanthis is one of the most important Chinese traditional medicines, is the fruits of *Trichosanthes kirilowii* Maxim and *Trichosanthes kirilowii* Harms[1]. It has various functions such as dilate coronary arteries, enforce the ability of antihypoxia, antibiosis, antiphlogosis, antitumous, reducing serum cholesterol[2-4]. Previous phytochemical investigation of this plant led to the isolation of triterpenoids, sterols, amino acids, volatile oils and so on[5-7]. Fruits and seeds of *Trichosanthes kirilowii* Maxim and *Trichosanthes kirilowii* Harms have embodied in the pharmacopoeia of China (2010) as medical parts, however there is no report on the twigs of *Trichosanthes kirilowii* Maxim. and *Trichosanthes kirilowii* Harms. In order to further develop and utilize the *Trichosanthes kirilowii* Maxim. and *Trichosanthes kirilowii* Harms, the study on chemical constituents of the twigs of *Trichosanthes kirilowii* Maxim. and *Trichosanthes kirilowii* Harms. is necessary.

In the present study, the ethyl acetate extract of the twigs of *Trichosanthes kirilowii* Maxim. was investigated. Here, we reported the isolation and identification of twelve compounds 4-[formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]-butanoic acid (**1**), 3-hydroxy-1-(4-hydroxyphenyl)-1-propanone (**2**), *p*-hydroxybenzoic acid (**3**), 4-(2-formyl-5-hydroxymethylpyrrol-1-yl) butyric acid (**4**), 2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-propane-1,3-diol (**5**), 2-[4-(3-hydroxy-1-propenyl)-2-methoxyphenoxy]-1,3-propanediol (**6**), (-)-methyl dihydrophaseate (**7**), 4'-dihydrophaseic acid (**8**), 4-hydroxy-3-methyl-oxybenzoic acid (**9**), quercetin-3-O-rutinoside (**10**), salicylic acid (**11**), 2-methoxy-2-(4'-hydroxyphenyl)-ethanol (**12**).

EXPERIMENTAL

All organic solvents used for extraction and separation are of analytical grade and purchased from Damao Chemical Factory (Tianjin, China). Methanol used for HPLC is HPLC grade and purchased from Tedia Company Inc., (Fairfield, USA) and water used is purified by Milli-Q water purification system (Millipore, Bedford, MA, USA).

Chromatography was carried out on silica gel 60 (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), ODS (40-75 mm, Fuji Silysia Chemical Ltd., Fuji Japan), MCI (YMC, Japan), Sephadex LH-20 (Pharmacia, Switzerland). The twigs of *Trichosanthes kirilowii* Maxim. were collected from Pingyin, Jinan, China and identified by Prof. Jia Li, Shandong University of Traditional Chinese Medicine, Jinan, Shandong, China.

HPLC analysis was carried out on an Agilent 1120 HPLC equipped with G4290A system, an auto sampler and a DAD detector (Agilent, California, USA). Preparative HPLC were carried out on a Shimadzu 20A HPLC system (Shimadzu, Kyoto, Japan). ESI-MS data were measured on an Agilent

1100LC/MSD mass spectrometer. (Agilent Corporation, USA). The ^{1}H and ^{13}C NMR and 2D NMR experiments were performed on a VARIAN INOVA-600 (Varian Corporation, USA) NMR spectrometer with tetramethyl silane (TMS) as internal standard.

Extraction and isolation: The twigs of *Trichosanthes kirilowii* Maxim. were crashed and extracted with EtOH-H_2O (9:1, v/v) by infiltrating. The combined extracts were concentrated under vacuum. The residue was suspended in H_2O and partitioned with petroleum ether (× 3) and EtOAc (× 3) in turn. The EtOAc layer (30 g) was subjected to MCI column chromatography with MeOH-H_2O (10, 30, 50, 70, 90 and 100 %) to obtained 7 fractions. All of the fractions were further purified by C-18 reversed-phase open column, Sephadex LH-20 and preparative HPLC to obtain compound **1** (15.7 mg), compound **2** (11.4 mg), compound **3** (20.5 mg), compound **4** (14.1 mg), compound **5** (12.3 mg), compound **6** (5.1 mg), compound **7** (5.2 mg), compound **8** (15.8 mg), compound **9** (14.5 mg), compound **10** (21.8 mg), compound **11** (13.2 mg) and compound **12** (14.3 mg).

RESULTS AND DISCUSSION

Compound 1: $C_{11}H_{15}NO_4$. ESI-MS *m/z*: 224[M-H]$^-$; ^{1}H NMR (CD_3OD, 600 Hz) δ: 9.44 (1H, s, H-1''), 6.98 (1H, d, *J* = 4.2 Hz, H-3), 6.27 (1H, d, *J*=4.2 Hz, H-4), 4.48 (2H, s, H-1'''), 4.36 (2H, m, H-1'), 3.51 (3H, s, H-2'''), 2.32 (2H, m, H-3'), 2.09 (2H, m, H-2'); ^{13}C-NMR(150 MHz, CD_3OD) δ:132.3 (C-2), 124.4 (C-3), 111.4 (C-4), 139.6 (C-5), 44.5 (C-1'), 26.3 (C-2'), 30.5 (C-3'), 175.4 (C-4'), 179.6 (C-1''), 64.9 (C-1'''), 56.8 (C-2'''). The spectra data were consistent with those reported in reference, so compound **1** was identified as 4-[formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]-butanoic acid[8].

Compound 2: $C_9H_{10}O_3$. ESI-MS *m/z*: 167 [M + H]$^+$, 189 [M + Na]$^+$; 165[M-H]$^-$. ^{1}H NMR(CD_3OD, 600 Hz) δ:7.89 (2H, d, *J* = 8.4 Hz, H-2',6'), 6.84 (2H, d, *J*=8.4 Hz, H-3',5'), 3.93 (2H, t, *J* = 6.0 Hz, H-2), 3.14 (2H, t, *J*=6.0 Hz, H-3); ^{13}C NMR (150 MHz, CD_3OD) δ:198.2 (C-1), 40.2 (C-2), 57.4 (C-3), 128.7 (C-1'), 130.4 (C-2'), 114.8 (C-3'), 162.5 (C-4'), 114.8 (C-5'), 130.4 (C-6'). The spectra data were consistent with those reported in reference, so compound **2** was identified as 3-hydroxy-1-(4-hydroxyphenyl)-1-propanone[9].

Compound 3: $C_7H_6O_3$. ESI-MS *m/z*: 137[M-H]$^-$. ^{1}H NMR (CD_3OD, 600 Hz) δ: 7.89 (2H, d, *J* = 8.4 Hz, H-2,6), 6.83 (2H, d, *J* = 9 Hz, H-3,5). ^{13}C NMR (150 MHz, CD_3OD) δ:122.0 (C-1), 131.6 (C-2), 114.6 (C-3), 161.8(C-4), 114.6 (C-5), 131.6 (C-6), 169.4 (C-7). The spectra data were consistent with those reported in reference, so compound **3** was identified as *p*-hydroxybenzoic acid[10].

Compound 4: $C_{10}H_{13}NO_4$. ESI-MS *m/z*: 234 [M + Na]$^+$, 445 [2M + Na]$^+$; 210 [M-H]$^-$. ^{1}H NMR (CD_3OD, 600 Hz) δ:9.42 (1H, s, H-6), 6.98 (1H, d, *J*=4.2 Hz, H-3), 6.26 (1H, d, *J* = .6 Hz, H-4), 4.64 (2H, s, H-7), 4.39 (2H, t, *J* = 7.8 Hz, H-4'), 2.32 (2H, t, *J* = 7.2 Hz, H-2'), 2.00 (2H, dt, *J* = 7.8, 7.2 Hz, H-3'). ^{13}C NMR (150 MHz, CD_3OD) δ: 132.0 (C-2), 124.5 (C-3), 110.0 (C-4), 143.2 (C-5), 178.0 (C-6), 54.9 (C-7), 30.4 (C-2'), 26.2 (C-3'), 44.4 (C-4'). The spectra data were consistent with those reported in reference, so compound **4** was identified as 4-(2-formyl-5-hydroxymethylpyrrol-1-yl)-butyric acid[11,12].

Compound 5: The ESI-MS of compound **5** showed a quasimolecular ion [M + Na]$^+$ at *m/z* 279. Taking into the 13 carbons displayed in its ^{13}C NMR spectra, the molecular formula was established as $C_{13}H_{20}O_5$. The ^{1}H NMR spectrum of 5 displayed a typical ABX aromatic proton system at δ_H 6.98 (1H, d, *J* = 8.4 Hz), 6.85 (1H, brs), 6.73 (1H, brd, *J* = 8.4 Hz), one methoxyl group at δ_H 3.77 (3H, s). The ^{13}C NMR spectrum of 5 confirmed the presence of one aromatic ring and one methoxyl group. The structural assignment was achieved by DEPT, HSQC, HMBC and gCOSY spectra. The HMBC correlations from H-8 to C-7, C-9 and C-1, H-7 to C-8, C-9, C-6, C-2 and C-1, H-9 to C-7, C-8 could be observed. The long range correlations from the methoxyl group to C-3 showed that the methoxyl group was attached to C-3. In the HMBC spectrum, the H-2' had cross-peaks with C-1', C-3' and C-4. From the above spectral data, compound **5** was deduced to be 2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-propane-1,3-diol[13].

ESI-MS *m/z*: 279 [M + Na]$^+$, 535 [2M + Na]$^+$. ^{1}H NMR (CD_3OD, 600 MHz) δ: 6.98 (1H, d, *J* = 8.4 Hz, H-5), 6.85 (1H, brs, H-2), 6.73 (1H, brd, *J* = 8.4 Hz, H-6), 4.42 (2H, m, H-1'), 4.14 (1H, m, H-2'), 3.77 (3H, s, 3-OCH_3), 3.72 (2H, m, H-3'), 3.55 (2H, brs, H-9), 2.62 (2H, m, H-7), 1.81 (2H, m, H-8). ^{13}C NMR (150 MHz, CD_3OD) δ: 136.8 (C-1), 112.6 (C-2), 150.5 (C-3), 145.3 (C-4), 117.9 (C-5), 120.4 (C-6), 31.2 (C-7), 34.1 (C-8), 60.7 (C-9), 60.5 (C-1'), 81.8 (C-2'), 60.5 (C-3'), 54.9 (3-OCH_3).

Compound 6: The ESI-MS of compound **6** showed a quasimolecular ion [M + Na]$^+$ at *m/z* 277. Taking into the 13 carbons displayed in its ^{13}C NMR spectra, the molecular formula was established as $C_{13}H_{18}O_5$. The ^{13}C NMR spectral data of 6 were similar with those of 5 except for C-7 and C-8 shifting downfield by 98.7 and 93.1ppm, respectively, suggesting that 6 have similar structure with 5. The relationship of H7 and H8 was *trans*, which was verified by the coupling constant (*J* = 15.6 Hz). From the above spectral data, compound **6** was deduced to be 2-[4-(3-hydroxy-1-propenyl)-2-methoxy-phenoxy]-1,3-propanediol[14].

ESI-MS *m/z*: 277 [M + Na]$^+$, 531 [2M + Na]$^+$. ^{1}H NMR (CD_3OD, 600 MHz) δ:7.06 (1H,brs, H-2), 7.02 (1H, d, *J* = 8.4 Hz, H-5), 6.94 (1H, d, *J*=7.8Hz, H-6), 6.54 (1H, d, *J* = 15.6Hz, H-7), 6.27 (1H, m, H-8), 3.75 (2H, m, H-9), 4.21 (1H, m, H-2'), 3.86 (3H, s, 3-OCH_3), 4.22(4H, m, H-1', H-3'). ^{13}C NMR (150 MHz, CD_3OD) δ:131.8 (C-1), 109.8 (C-2), 150.5 (C-3), 147.0 (C-4), 117.2 (C-5), 119.3 (C-6), 129.9 (C-7), 127.2 (C-8), 62.3 (C-9), 60.5 (C-1'), 81.4 (C-2'), 60.5 (C-3'), 55.0 (3-OCH_3).

Compound 7: The ESI-MS of compound **7** showed a quasimolecular ion [M+Na]$^+$ at *m/z* 305. Taking into account the ^{1}H and ^{13}C NMR spectra, the molecular formula was established as $C_{15}H_{22}O_5$. ^{1}H NMR spectrum of compound **7** displayed three methyl groups at δ_H 0.93 (3H, s), 1.13 (3H, s), 2.07 (3H, s), an olefinic proton signal δ_H 5.76 (1H, brs) and a pair of protons at δ_H 7.95 (1H, d, *J* = 16.2 Hz) and 6.49 (1H, d, *J* = 16.2 Hz) in a trans-disubstituted double bond. The ^{13}C and DEPT NMR spectra of **7** showed 15 carbon signals classified as 3 methyls, three methylenes, four methines, three quaternary carbons and one carbonyl carbons. The structural assignment

was achieved by DEPT, HSQC, HMBC and gCOSY spectra. In the HMBC spectrum, the methyl proton at δ_H 2.07 (3H, s) was found to have clear correlation with C-8, C-9, C-10, H-10 was found to have clear correlation with C-8, C-11, C-15. The HMBC correlations from H-13 to C-2, C-3, C-6 and C-12, H-14 to C-3, C-4, C-5 and C-6, H-7 to C-6, C-8, C-9 and C-10 could be observed. From the above spectral data, compound **7** was deduced to be 4'-dihydrophaseic acid[16].

ESI-MS *m/z*: 305[M+Na]$^+$, 587[2M + Na]$^+$, 281[M-H]$^-$. ^{1}H NMR (600 MHz,CD_3OD) δ: 7.95 (1H, d, *J* = 16.2Hz, H-8), 6.49 (1H, d, *J* = 16.2Hz, H-7), 5.76 (1H, brs, H-10), 3.79 (1H, d, *J* = 7.2Hz, H-12), 3.70 (1H, d, *J* = 7.2Hz, H-12), 2.02 (1H, m, H-4), 1.84 (1H, m, H-4), 2.07 (3H, s), 1.72 (1H, dd, *J* = 12Hz, 7.2Hz, H-2), 1.64 (1H, dd, *J* = 12Hz, 1.8Hz, H-2), 1.13 (3H, s, H-14), 0.93 (3H, s, H-13). ^{13}C NMR (150 MHz, CD_3OD) δ:47.1 (C-1), 43.1 (C-2), 64.6 (C-3), 44.5 (C-4), 86.4 (C-5), 81.8 (C-6), 133.2 (C-7), 130.5 (C-8), 148.9 (C-9), 118.8 (C-10), 169.5 (C-11), 75.8 (C-12), 14.9 (C-13),18.2 (C-14), 19.7 (C-15).

Compound 8: The ESI-MS of Compound **8** showed a quasimolecular ion [M+Na]$^+$ at *m/z* 295. Taking into the 16 carbons displayed in its ^{13}C NMR spectra, the molecular formula was established as $C_{16}H_{24}O_5$. The ^{13}C NMR spectral data of compound **8** were similar with those of compound **7** except for the presence of a methoxy group, indicating that they had the same molecular skeleton. From the above spectral data, compound **8** was deduced to be (-)-methyl dihydrophaseate[15].

ESI-MS *m/z*: 295[M-H]$^-$. ^{1}H NMR (600 MHz,CD_3OD) δ: 7.93 (1H, d, *J* = 15Hz, H-8), 6.34 (1H, d, *J* = 16.2Hz, H-7), 5.87 (1H, brs, H-10), 3.76 (1H, d, *J* = 7.2Hz, H-12), 3.70 (1H, d, *J* = 7.2Hz, H-12), 1.90 (1H, m, H-4), 1.73 (1H, m, H-4), 2.04 (3H, brs, H-15), 1.85 (1H, dd, *J* = 13.8Hz, 7.2Hz, H-2), 1.60 (1H, m, H-2), 1.31 (3H, s, H-14), 1.07 (3H, s, H-13), 3.42 (3H, s, -OCH_3). ^{13}C NMR (150 MHz, CD_3OD) δ: 47.2 (C-1), 39.5 (C-2), 63.8 (C-3), 40.8 (C-4), 88.5 (C-5), 81.3 (C-6), 132.2 (C-7), 128.9 (C-8), 151.9 (C-9), 116.5 (C-10), 167.3 (C-11), 74.2 (C-12), 13.1 (C-13),17.1 (C-14), 19.3 (C-15), 52.0 (-OCH_3).

Compound 9: $C_8H_8O_4$. ESI-MS *m/z*: 167 [M-H]$^-$. ^{1}H NMR (600 MHz, CD_3OD) δ: 7.56 (1H, d, *J* = 8.4Hz, H-6), 7.54 (1H, s, H-2), 6.83 (1H, d, *J* = 8.4Hz, H-5), 3.88 (3H, s, 4-OCH_3). ^{13}C NMR (150 MHz, CD_3OD) δ: 123.7 (C-1), 114.3 (C-2), 150.8 (C-3), 147.1 (C-4), 112.4 (C-5), 122.7 (C-6), 169.5 (C-7), 54.9 (-OCH_3). The spectra data were consistent with those reported in reference, so compound **9** was identified as 4-hydroxy-3-methyloxybenzoic acid[17].

Compound 10: $C_{20}H_{18}O_{11}$. ESI-MS *m/z*: 433 [M-H]$^-$. ^{1}H NMR (600 MHz, CD_3OD) δ: 12.65 (1H, s, H-5), 7.67 (1H, d, *J* = 7.8Hz, H-2'), 7.52 (1H, brs, H-6'), 6.85 (1H, d, *J* = 7.8Hz, H-5'), 6.39 (1H, brs, H-8), 6.18 (1H, s, H-6), 5.28 (1H, d, *J* = 5.4 Hz, H-1"). ^{13}C NMR (150 MHz, CD_3OD) δ: 156.5 (C-2), 134.1 (C-3), 177.8 (C-4), 161.6 (C-5), 99.3 (C-6), 165.4 (C-7), 94.0 (C-8), 156.5 (C-9), 104.0 (C-10), 121.2 (C-1'), 115.8 (C-2'), 145.5 (C-3'), 149.2 (C-4'), 116.1 (C-5'), 122.4 (C-6'), 101.8 (C-1"), 72.1 (C-2"), 71.1 (C-3"), 66.5 (C-4"), 64.7 (C-5"). The spectra data were consistent with those reported in reference, so compound **10** was identified as quercetin-3-O-rutinoside[18].

Compound 11: $C_7H_6O_3$. ESI-MS *m/z*: 275[2M-1]$^-$. ^{1}H NMR (600 MHz, CD_3OD) δ: 7.85 (1H, d, *J* = 6.6 Hz, H-6), 7.42 (1H, t, *J* = 6.6 Hz, H-4), 6.88 (2H, m, H-3, H-5). ^{13}C NMR (150 MHz, CD_3OD) δ: 111.8 (C-1), 161.7 (C-2), 116.5 (C-3), 134.7 (C-4), 118.4 (C-5), 130.1 (C-6), 173.8 (C-7). The spectra data were consistent with those reported in reference, so compound **11** was identified as salicylic acid[19,20].

Compound 12: $C_9H_{12}O_3$. ESI-MS *m/z*: 191 [M + Na]$^+$, 169 [M + H]$^+$. ^{1}H NMR (600 MHz, CD_3OD) δ: 3.30 (3H, s, 2-OCH_3), 3.80 (1H, d, *J* = 7.8 Hz, H-1), 4.19 (1H, dd, *J* = 7.2 Hz, 11.4Hz, H-1), 4.68 (1H, brs, H-2), 6.76 (2H, d, *J* = 7.8 Hz, H-3', H-5'), 7.19 (2H, d, *J* = 7.8 Hz, H-2', H-6'). ^{13}C NMR (150 MHz, CD_3OD) δ: 71.1 (C-1), 85.9 (C-2), 114.7 (C-3', C-5'), 127.2 (C-2', C-6'), 131.5 (C-1'), 156.8 (C-4'), 53.8 (2-OCH_3). The spectra data were consistent with those reported in reference, so compound **12** was identified as 2-methoxy-2-(4'-hydroxyphenyl)ethanol[21].

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