Chemical Constituents from *Inula Japonica* Thunb

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ABSTRACT: OBJECTIVE To study the chemical constituents in *Inula japonica* Thunb. **METHODS** A variety of separation technologies were used for the isolation of compounds, and their structures were identified by physical properties and spectroscopic techniques (NMR, IR, UV and MS). **RESULTS** Eleven compounds, chrysoeriol(Ⅰ), patuletin(Ⅱ), quercetin(Ⅲ), luteolin(Ⅳ), kaempferol(Ⅴ), inuchinenolide C(Ⅵ), britannilactone(Ⅶ), daucosterol(Ⅷ), β-sitosterol(Ⅸ), britanin(Ⅹ) and ivangustin(Ⅺ), were obtained from *Inula japonica* Thunb. **CONCLUSION** Compound Ⅰ was isolated from this genus for the first time.

KEY WORDS: *Inula japonica* Thunb; chemical constituents; extraction and separation; identification; spectroscopic techniques

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旋覆花化学成分研究

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摘要：目的 研究旋覆花 *Inula japonica* Thunb.的化学成分。**方法** 采用多种分离技术对旋覆花乙醇提取物的氯仿和乙酸乙酯萃取部分分离纯化，根据其理化常数测定和光谱数据进行结构鉴定。**结果** 从旋覆花中分得 11 个化合物，经鉴定为金圣草素(Ⅰ)，万寿菊素(Ⅱ)，槲皮素(Ⅲ)，木犀草素(Ⅳ)，山柰酚(Ⅴ)，旋覆花内酯 C(Ⅵ)，旋覆花内酯(Ⅶ)，胡萝卜苷(Ⅷ)，β-谷甾醇(Ⅸ)，大花旋覆花内酯(Ⅹ)，ivangustin(Ⅺ)。**结论** 化合物Ⅰ为首次从欧亚旋覆花植物中得到。

关键词：旋覆花；化学成分；提取分离；结构鉴定；光谱技术

中图分类号：R284.1　　**文献标志码：**B　　**文章编号：**1007-7693(2011)04-0330-05

Inula japonica Thunb, belonging to the family Compositae, which dry capitulum can be used medicinally for anti-inflammatory, antibacteria, allaying asthma, relieving cough, anti-hepatitis, anti-tumor and so on[1]. The chemical constituents in *I. japonica* have been widely reported so far[2-5]. In this study, eleven compounds were isolated and identified as chrysoeriol(Ⅰ), patuletin(Ⅱ), quercetin (Ⅲ), luteolin (Ⅳ), kaempferol(Ⅴ), inuchinenolide C(Ⅵ), britannilactone(Ⅶ), daucosterol(Ⅷ), β-sitosterol(Ⅸ), britanin(Ⅹ), and ivangustin (Ⅺ) (shown in Table 1). Except compounds Ⅲ, Ⅶ, Ⅺ, the others were isolated from the plant for the first time and compound Ⅰ was isolated from this genus for the first time.

1　Materials and methods

1.1　Experimental material

All melting points were determined using a micro melting point apparatus (Beijing Tech Instrument Co., Ltd) and were uncorrected. The ^{1}H- and ^{13}C-NMR spectra were recorded on the INOVA-500 NMR spectrometer. The MS data were recorded on VGZAB-2F, Micromass UK GCT-MS, Shimadzu JP GCMS-QP5050 A mass spectrometer. Commercial silica gel (200-300 mesh) and silica gel GF254(40 μm) (Qingdao Haiyang Chemical Group Co. Ltd) was used for column chromatography and thin layer chromatography respectively. Polyamide (80 mesh) was provided by Zhejiang Huangyan Chemical Company; Sephadex LH-20 was made by Sweden Pharmacia Biotech, Sueden company; DMSO was provided by Beijing Tongzheng Biological Company. Other reagents were analytical grade.

1.2　Plant material

Samples of *Inula japonica* Thunb in this experiment were purchased from Tongrentang Co., Ltd. in Beijing, batch number: 050201. Samples were identified as *Inula japonica* by Professor LU Jincai of Shenyang Pharmaceutical University.

1.3　Extraction and isolatin

The dry capitulum of *I. japonica* (5 kg), were refluxed with 80% ethanol for 5 h and filtered. The filtrate was concentrated into about 5 L and successively extracted with petroleum ether(60–90℃, $CHCl_3$, EtOAc, *n*-butanol). Condensed into condensate respectively. The $CHCl_3$-soluble part(30 g) was subjected to column chromatography over silica gel eluted with petroleum ether-acetone gradient (ratio 10∶1 to 0∶1), then purified with Sephadex LH-20 column chromatography and recrystallized to obtain chrysoeriol(Ⅰ), inuchinenolide C(Ⅵ), britannilactone(Ⅶ), daucosterol(Ⅷ), britanin(Ⅹ), and ivangustin(Ⅺ); EtOAc-soluble part(83 g) was subjected to column chromatography over silica gel eluted with petroleum $CHCl_3$-methanol gradient (1%–100% methanol), then purified with Sephadex LH-20 column chromatography and recrystallized to obtain patuletin(Ⅱ), quercetin(Ⅲ), luteolin(Ⅳ), kaempferol(Ⅴ), β-sitosterol(Ⅸ), as shown in Fig 1, Fig 2.

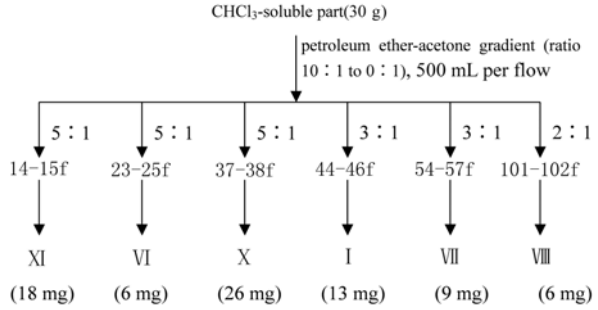
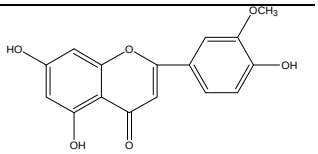
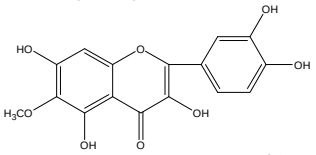
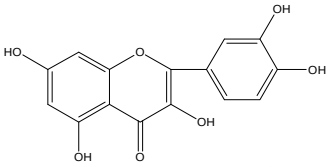
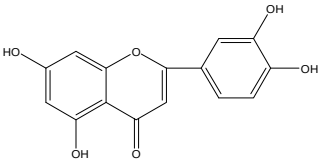
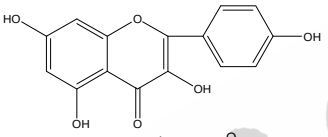
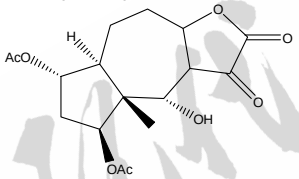
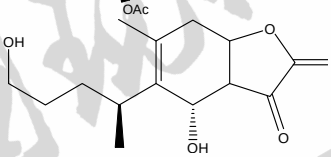
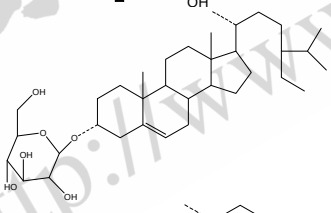
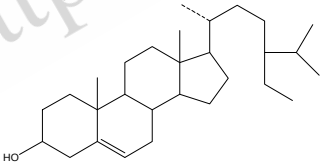
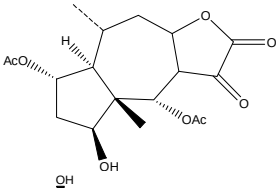
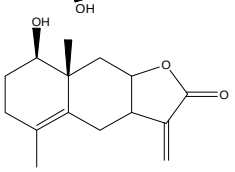


Fig 1　$CHCl_3$-soluble part seperation figure

图 1　氯仿法分离图

Tab 1 Name, formula and structure of compounds
表 1 化合物名称、分子式和结构式

Compound	Formula	Structure
chrysoeriol(Ⅰ)	$C_{16}H_{12}O_6$	
patuletin(Ⅱ)	$C_{16}H_{12}O_8$	
quercetin(Ⅲ)	$C_{15}H_{10}O_7$	
luteolin(Ⅳ)	$C_{15}H_{10}O_6$	
kaempferol(Ⅴ)	$C_{15}H_{10}O_6$	
inuchinenolide C(Ⅵ)	$C_{19}H_{24}O_7$	
britannilactone(Ⅶ)	$C_{15}H_{22}O_4$	
daucosterol(Ⅷ)	$C_{35}H_{49}O_6$	
β-sitosterol(Ⅸ)	$C_{29}H_{50}O$	
britanin(Ⅹ)	$C_{19}H_{24}O_7$	
ivangustin(Ⅺ)	$C_{15}H_{20}O_3$	

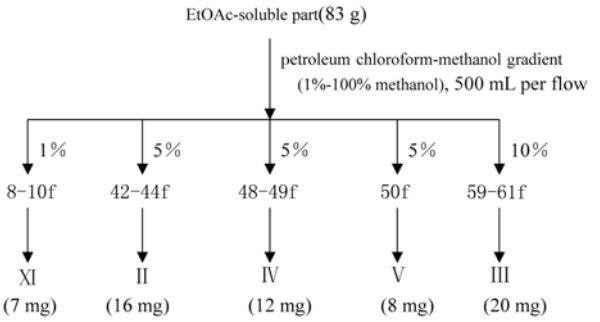


Fig 2 EtOAc-soluble part separation figure
图 2 乙酸乙酯法分离图

2 Characterization

2.1 4',5,7-Trihydroxy-3'-methoxyflavone[chrysoeroil(Ⅰ)]

Yellow-green needles(DMSO), mp 326–328 ℃, HCl-Mg reaction positive, ESI-MS(*m/z*): 301$[M+H]^+$, 299$[M-H]^-$, 286$[M+H-CH_3]^+$, 258$[M+H-CH_3-CO]^+$. ^{1}H-NMR(DMSO-d_6, 500 MHz)δ: 12.95(1H, s, 5-OH), 7.54(2H, s, H-2', H-6'), 6.92(1H, d, *J*=9 Hz, H-5'), 6.88(1H, s, H-3), 6.49(1H, s, H-8), 6.18(1H, s, H-6), 3.88(3H, s, -OCH_3). ^{13}C-NMR(DMSO, 125 MHz)δ: 181.8(C-4), 164.3(C-7), 163.6(C-2), 161.4(C-9), 157.3(C-5), 150.7(C-3'), 148.0 (C-4'), 121.5(C-6'), 120.3(C-1'), 115.8(C-5'), 110.2(C-2'), 103.6(C-3), 103.2(C-10), 98.8 (C-6), 94.0(C-8), 55.9(3'-OCH_3). The above data was consistent with the reference[6].

2.2 7-[β-D-Glucopyranosyl]oxy]-3,3',4',5-tetrahydroxy-6-methoxyflavone [patuletin(Ⅱ)]

Yellow needles(CH_3OH), mp 244–247 ℃, HCl-Mg reaction positive, EI-MS (*m/z*): 332$[M]^+$, 314$[M-H_2O]^+$, 289$[M-CH_3CO]^+$, ^{1}H-NMR(CD_3COCD_3, 500 MHz)δ: 12.32(1H, s, 5-OH), 7.82 (1H, d, *J*=2 Hz, H-2'), 7.69(1H, dd, *J*=2, 8.5 Hz, H-6'), 6.98(1H, d, *J*=8.5 Hz, H-5'), 6.59(1H, s, H-8), 3.87 (3H, s, -OCH_3). ^{13}C-NMR(CD_3COCD_3, 125 MHz)δ: 176.8(C-4), 157.8(C-7), 153.0(C-5), 152.5(C-9), 148.3(C-4'), 147.1(C-2), 145.8 (C-3'), 136.4(C-3), 131.6(C-6), 123.8(C-1'), 121.4(C-6'), 116.2(C-5'), 115.8(C-2'), 104.5 (C-10), 94.4(C-8), 60.7(6-OCH_3). The above data was identical with the literature data[7-8].

2.3 3,3',4',5,7-Pentahydroxyflavone [quercetin(Ⅲ)]

Yellow powder(CH_3OH), mp 313–314 ℃, HCl-Mg reaction positive. EI-MS(*m/z*): 302$[M]^+$. ^{1}H-NMR (CD_3COCD_3, 500 MHz)δ: 2.16(1H, s, 5-OH), 7.82 (1H, H-2'), 7.69(1H, dd, *J*=8.5 Hz, H-6'), 6.99(1H, d,

J=8 Hz, H-5'), 6.52(1H, H-8), 6.26(1H, H-6). ^{13}C-NMR(CD_3COCD_3, 125 MHz)δ: 165.2(C-7), 162.2 (C-9), 157.7(C-5), 148.4(C-4'), 147.0(C-2), 145.9 (C-3'), 123.6(C-1'), 121.4(C-6'), 116.1(C-5'), 115.6(C-2'), 104.0(C-10), 99.1(C-6), 94.4(C-8). The above data was identical with the literature data[7-8].

2.4 3',4',5,7-Tetrahydroxyflavone [luteolin(Ⅳ)]

Yellow graininess(CH_3OH), mp 203–205 ℃, HCl-Mg reaction positive. EI-MS(*m/z*): 286$[M]^+$, 258$[M-CO]^+$. ^{1}H-NMR(C_5D_5N, 500 MHz)δ: 7.89 (1H, d, *J*=1.5Hz, H-2'), 7.54(1H, dd, *J*=1.5, 8 Hz, H-6'), 7.27(1H, d, *J*=8.5 Hz, H-5'), 6.92(1H, H-6), 6.72(2H, H-3, H-8). ^{13}C-NMR(C_5D_5N, 125 MHz)δ: 182.7(C-4), 165.8(C-7), 164.8(C-2), 163.2(C-9), 158.5(C-5), 151.7(C-4'), 147.8(C-3'), 123.0 (C-1'), 119.5(C-6'), 116.8(C-5'), 114.6(C-2'), 105.0(C-10), 104.0(C-3), 99.9(C-6), 94.8(C-8). The above data was identical with the literature data[9].

2.5 3, 5, 7, 4'-Tetrahydroxyflavonoid [kaempeferol(Ⅴ)]

Yellow needles(CH_3OH), mp 272–273 ℃. Polyamide-TLC-one yellow spot, 1% $AlCl_3$-CH_3OH-bright yellow, HCl-Mg reaction positive. EI-MS(*m/z*): 286$[M]^+$, 258$[M-CO]^+$. ^{1}H-NMR(CD_3COCD_3, 500 MHz)δ: 12.16(1H, s, 5-OH), δ9.64(1H, s, 7-OH), δ8.99(1H, s, 4'-OH), δ7.99(1H, s, 3-OH), δ8.15(2H, d, *J*=9 Hz, H-2', H-6'), δ7.01(2H, d, *J*=9 Hz, H-3', H-5'), δ6.53(1H, d, *J*=1.5 Hz, H-8), δ6.26 (1H, d, *J*=2.0 Hz, H-6). The above data was identical with the literature data [10].

2.6 Inuchinenolide C (Ⅵ)

Colourless tabular crystal ($CHCl_3$), mp 156–158 ℃, $[\alpha]_D^{20}$ -25.9°(c 1.13, $CHCl_3$). EI-MS(*m/z*): 246$[M-2\times HOAC]^+$, 228$[M-2\times HOAC-H_2O]^+$. ^{1}H-NMR($CDCl_3$, 500 MHz)δ: 6.20(1H, d, *J*=3.5 Hz, H-13'), 5.98(1H, d, *J*=3 Hz, H-13), 5.61(1H, dd, *J*=8.5, 9 Hz, H-4), 4.94(1H, br t, *J*=8 Hz, H-2), 4.87(1H, br s, 6-OH), 4.45(1H, m, H-8), 3.52(1H, dd, *J*=6.5 Hz, H-6), 2.14, 2.04(3H, s, 2, 4-$OCOCH_3$), 0.99(1H, d, *J*=6 Hz, 14-CH_3), 0.91(3H, s, 15-CH_3). ^{13}C-NMR($CDCl_3$, 125 MHz)δ: 173.2, 170.2(2, 4-$OCOCH_3$), 170.0(C-12), 139.5(C-11), 121.7 (C-13), 76.2(C-2), 76.0(C-4), 75.0(C-6), 74.5(C-8), 53.9(C-7), 52.3(C-5), 50.0(C-1), 44.2(C-3), 35.3(C-9), 30.4 (C-10), 21.4(2CH3COO-), 20.8(C-15), 17.3(C-14). The above data was identical with the literature data[11].

2.7 Britannilactone (Ⅶ)

Colourless tabular crystal ($CHCl_3$), mp 175–177 ℃, $[\alpha]_D^{20}$ +91.3°(c 0.092, $CHCl_3$). EI-MS(*m/z*): 266$[M]^+$, 248$[M-H_2O]^+$, 233$[M-H_2O-CH_3]^+$, 230$[M-2\times H_2O]^+$, 215$[M-2\times H_2O-CH_3]^+$, 189$[M-2\times H_2O-C_3H_5]^+$。^{1}H-NMR($CDCl_3$, 500 MHz)δ: 6.32(1H, d, *J*=2 Hz, H-13), 5.78(1H, d, *J*=3 Hz, H-13'), 5.38 (1H, ddd, *J*=2, 1.5, 2 Hz, H-8), 4.15(1H, d, *J*=2 Hz, H-6), 3.53(2H, m, H-1, H-7), 3.40(1H, m, H-1'), 2.84(1H, dd, *J*=2.5, 16 Hz, H-9β), 2.70(1H, m, H-4), 2.48(1H, dd, *J*=2.5, 16 Hz, H-9α), 1.76(3H, d, *J*=1 Hz, H-14), 1.30(3H, m, H-2, H-2', H-3), 1.08(3H, d, *J*=7 Hz, H-15), 1.04(1H, m, H-3'). ^{13}C-NMR($CDCl_3$, 125 Hz) δ: 170.9(C-12), 137.0(C-5), 136.7(C-11), 131.5 (C-10), 124.7(C-13), 76.6(C-8), 68.7(C-6), 62.8(C-1), 45.4(C-7), 34.3(C-9), 33.2(C-4), 31.4(C-3), 31.2 (C-2), 20.5(C-14), 19.5(C-15). The above data was identical with the literature data[12-13].

2.8 Daucosterol (Ⅷ)

White tabular crystal(CH_3OH-$CHCl_3$), mp 290–292 ℃. FAB-MS(*m/z*): 413$[M-C_6H_{11}O_5]^+$, 397 [M-Gly-]$^+$. ^{1}H-NMR(C_5D_5N, 500 MHz)δ: 5.34(1H, s, H-6), 5.04(1H, d, *J*=8.5 Hz, H-1'), 2.74(1H, d, *J*=10.5 Hz, H-4'), 2.47(1H, t, *J*=11.5, 12 Hz, H-4). ^{13}C-NMR(C_5D_5N, 125 MHz)δ: 141.3(C-5), 122.3 (C-6), 102.9 (C-1'), 79.0(C-5'), 78.8(C-3'), 78.4(C-3), 75.7(C-2'), 72.7(C-4'), 63.2(C-6'), 57.2(C-14), 56.6(C-17), 50.7(C-9), 46.4(C-24), 42.8(C-13), 40.3(C-12), 39.7(C-4), 37.8(C-1), 37.3(C-10), 36.7(C-20), 34.6(C-22), 32.5(C-7), 32.4(C-8), 30.6(C-2), 29.8(C-25), 28.9(C-16), 26.8(C-23), 24.9(C-15), 23.8(C-28), 21.6(C-11), 20.3(C-27), 19.8(C-26), 19.6(C-19), 19.4(C-21), 12.9(C-29), 12.5(C-18). The above data was identical with the literature data[14].

2.9 β-Sitosterol (Ⅸ)

White needles(petroleum-ether), mp 139–140 ℃. Liebermann-Burchard reaction positive. Color reaction and R_f values on TCL are consistent with those of authentic sample β-sitosterol.

2.10 Tanin(Ⅹ)

Colorless prismatic crystal ($CHCl_3$/C_2H_5OH), mp 188-190℃, $[\alpha]_D^{20}$ -26°(c 5.0, $CHCl_3$). UV_{254} absorption. EI-MS(*m/z*): 246$[M-2\times HOAc]^+$, 228

[M-2×HOAc-H_2O]$^+$. ^{1}H-NMR($CDCl_3$, 500 MHz)δ: 6.14(1H, t, *J*=2, 1.5 Hz, H-13'), 5.36 (1H, s, H-13), 5.04(1H, d, *J*=8.5 Hz, H-6), 4.87(1H, dd, *J*=6, 9 Hz, H-6), 4.50(1H, t, *J*=10, 11.5 Hz, H-8), 4.11 (1H, t, *J*=9, 10.5 Hz, H-4), 3.01(1H, t, *J*=9, 9.5 Hz, H-7), 2.41(2H, H-9', 4-OH), 2.22, 2.02(3H, s, 2, 4-$OCOCH_3$), 2.05(1H, H-3), 1.89(2H, m, H-1, H-10), 1.79(1H, dd, *J*=8.5, 15 Hz, H-3'), 1.42(1H, q, *J*=12, 12, 12 Hz, H-9), 1.00(3H, s, H-15), 0.96(3H, d, *J*=5 Hz, H-14). ^{13}C-NMR($CDCl_3$, 125 MHz)δ: 172.4, 170.2 (2, 4-$OCOCH_3$), 168.8(C-12), 138.4(C-11), 120.0 (C-13), 76.3(C-6), 76.1(C-8), 75.2(C-2), 72.8(C-4), 52.2(C-7), 51.0, 50.9(C-1, 5), 43.9(C-9), 36.2(C-3), 30.0(C-10), 21.1(2 × CH_3COO), 20.3(C-15), 15.8 (C-14). The above data was identical with the literature data[15].

2.11 Hydroxy-7αH, 8αH-eudesma-4, 11(13)-dien-8, 12-olide [ivangustin(Ⅺ)].

Colorless needles($CHCl_3$)，colorless prismatic crystal(CH_3OH-$CHCl_3$-H_2O)，mp 120−122 ℃, $[\alpha]_D^{27}$ + 85°(c 1.05, $CHCl_3$). UV254 absorption. EI-MS(m/z): 248[M]$^+$, 230[M-H_2O]$^+$, 204[M-OCO]$^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz)δ: 6.27(1H, d, *J*=3.5 Hz, H-13), 5.61(1H, d, *J*=2.5 Hz, H-13'), 4.48(1H, ddd, *J*=4.5, 4, 2 Hz, H-8α), 3.56(1H, dd, *J*=5, 11.5 Hz, H-1α), 3.06(1H, m, H-7α), 2.84(1H, dd, *J*=7.5, 13.5 Hz, H-6α), 2.27(1H, dd, *J*=5, 14 Hz, H-9β), 2.09(2H, m, H-3α, H-3β), 1.98(1H, t, *J*=12, 12.5 Hz, H-6β), 1.78(2H, m, H-2α, H-2β), 1.64(3H, s, H-15), 1.49(1H, dd, *J*=11, 14 Hz, H-9α), 1.02(3H, s, H-14). ^{13}C-NMR($CDCl_3$, 125 MHz): 170.8(C-12), 139.6 (C-11), 130.3(C-4), 126.5(C-5), 122.1(C-13), 75.9 (C-1), 71.9(C-8), 40.4(C-7), 39.1(C-10), 37.4(C-9), 30.9(C-2), 27.7(C-6), 27.0(C-3), 20.3(C-15), 18.9 (C-14). ^{1}H-NMR data was in accordance with the literature data[16] mainly, ^{13}C-NMR and MS data were identical with 8-epi-ivangustin [17].

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