

## Flavonol and monoterpene glycosides from *Ligularia macrophylla*

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**ABSTRACT: OBJECTIVE** To investigate the chemical constituents of the roots and rhizomes of *Ligularia macrophylla*. **METHODS** The compounds were isolated through repeated column chromatography on silica gel, polyamide, RP-18 and Sephadex LH-20. And the structures were elucidated on the basis of spectroscopic data (UV, IR, MS, <sup>1</sup>H NMR, <sup>13</sup>C NMR, DEPT and 2D NMR). **RESULTS** Four flavonol glycosides and two monoterpene glycosides were obtained and determined as rhamnazin-3-O-β-D-rutinoside (1), rhamnetin-3-O-β-D-rutinoside (2), rutin (3), afzelin (4), betulalbuside A (5) and 3, 7-dimethyloct-1-en-3, 8-diol-8-O-β-D-glucopyranoside (6). **CONCLUSION** All the compounds were isolated from this plant for the first time. **KEY WORDS:** *Ligularia macrophylla*; composita; flavonol glycosides; monoterpene glycosides

## 大叶橐吾中的黄酮醇苷和单萜苷

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**摘要:**目的 研究大叶橐吾的化学成分。方法 应用多种色谱方法和色谱材料进行提取、分离和纯化, 用各种现代光谱方法解析结构。结果 从大叶橐吾根茎的乙醇提取物中分离纯化到四个黄酮醇苷和两个单萜苷, 分别为甲基鼠李素-3-O-β-D-芸香糖苷(1), 鼠李素-3-O-β-D-芸香糖苷(2), 芦丁(3), 阿福豆苷(4), 梓木苷(5)和 3, 7-二甲基-1-辛-3, 8-二羟基-8-O-β-D-葡萄糖苷(6)。结论 所有化合物均为首次从该种植物中分离纯化得到。

**关键词:** 大叶橐吾; 菊科; 黄酮醇苷; 单萜糖苷

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### Introduction

*Ligularia macrophylla* DC. is a traditional Chinese medicinal herb used as antitussives. Pharmacologic study revealed that the crude extract of the plant exhibits antibacterial and antineoplastic activities as well as relieving cough and eliminating the phlegm<sup>[1]</sup>. So far, there are only a few reports about its chemical constituents<sup>[2-4]</sup>. Our phytochemical investigation on the ethanolic extract of its roots and rhizomes resulted in the isolation of six compounds including four flavonol glycosides, rhamnazin-3-O-β-D-rutinoside (1), rhamnetin-3-O-β-D-rutinoside (2), quercetin-3-O-β-D-rutinoside (3), kaempferol-3-O-α-L-rhamnopyranoside (4), and two monoterpene glycosides, betulalbuside A (5) and 3, 7-dimethyloct-1-en-3, 8-diol-8-O-β-D-glucopyranoside (6). All the compounds were firstly identified from the title plant. This paper deals with the isolation and characterization of

the six glycosides.

### Plant material

The plant was collected from Lijiang county, Yunnan province, China, in August 2001 and taxonomically authenticated by Prof. Peng Hua from Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 20010820ZY010) was deposited at the Herbarium of the Department of Pharmacognosy, College of Pharmaceutical Sciences, Zhejiang University.

### General experimental

Melting points were observed on an X-4 digital apparatus and are uncorrected. Optical rotations were measured with a Polax-2L polarimeter. UV spectra were recorded with a UV-1600 spectrophotometer. IR spectra were recorded as KBr pellets with a Bruker Vector-22 spectrometer. MS spectra were examined on

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an Esquire-LC-00075 mass spectrometer. NMR spectra were run on an INOVA-400 instrument with TMS as an internal standard.

Extraction and isolation

The air dried and powdered roots and rhizomes of *L. macrophylla* (2.0 kg) was extracted with 95% ethanol (5L × 4) at room temperature. Evaporation of the solvent under reduced pressure gave the crude extract (240 g), which was suspended in water and partitioned with petroleum ether, ethyl acetate and *n*-butanol, successively. The *n*-BuOH extract (80 g) was chromatographed on silica gel (1.0 kg) eluted with CHCl<sub>3</sub>-MeOH at increasing polarities to yield ten fractions. Further separation of fraction VII by polyamide and the Sephadex LH-20 column chromatography afforded compounds 1 and 2. Compound 3 was crystallized from fraction VIII and purified through recrystallization in MeOH. Compound 4 was obtained from fraction III by preparative TLC plates developed by CHCl<sub>3</sub>-MeOH (5:1). Fraction V and VI were repeatedly chromatographed on silica gel, RP-18 and Sephadex LH - 20 columns to give compounds 5 and 6,

Tab 1 <sup>13</sup>C NMR data (δ in DMSO-*d*<sub>6</sub>) for compounds 1-6

表 1 化合物 1-6的碳谱数据 (氘代 DMSO, δ)

carbon	1	2	3	4*	5	6
1					111.2 (t)	111.0 (t)
2	156.6 (s)	156.7 (s)	156.8 (s)	157.9 (s)	146.3 (d)	146.6 (d)
3	133.5 (s)	133.6 (s)	133.5 (s)	135.6 (s)	71.6 (s)	71.7 (s)
4	177.7 (s)	177.6 (s)	177.5 (s)	179.2 (s)	42.1 (t)	42.7 (t)
5	161.1 (s)	161.2 (s)	161.5 (s)	163.1 (s)	22.2 (t)	21.0 (t)
6	98.2 (d)	98.2 (d)	99.3 (d)	99.5 (d)	128.1 (d)	33.7 (t)
7	165.3 (s)	165.3 (s)	165.5 (s)	165.0 (s)	131.5 (s)	33.3 (d)
8	92.6 (d)	92.4 (d)	94.1 (d)	94.5 (d)	73.8 (t)	74.4 (t)
9	157.1 (s)	157.6 (s)	156.8 (s)	158.4 (s)	27.9 (q)	27.9 (q)
10	105.2 (s)	105.3 (s)	103.9 (s)	105.7 (s)	14.0 (q)	17.1 (q)
1'	121.1 (s)	120.0 (s)	121.4 (s)	122.4 (s)		
2'	113.6 (d)	115.8 (d)	115.6 (d)	131.6 (d)		
3'	149.9 (s)	145.9 (s)	145.2 (s)	116.3 (d)		
4'	147.2 (s)	151.0 (s)	148.9 (s)	160.9 (s)		
5'	115.5 (d)	116.4 (d)	116.5 (d)	116.3 (d)		
6'	122.7 (d)	122.1 (d)	121.9 (d)	131.6 (d)		
7-OMe	56.3 (q)	56.3 (q)				
3'-OMe	55.9 (q)					
glc						
1''	101.3 (d)	101.6 (d)	101.6 (d)		101.7 (d)	103.5 (d)
2''	74.5 (d)	74.4 (d)	74.4 (d)		73.6 (d)	73.7 (d)
3''	76.6 (d)	76.8 (d)	76.8 (d)		77.1 (d)	77.0 (d)
4''	70.4 (d)	70.3 (d)	70.3 (d)		70.4 (d)	70.4 (d)
5''	76.2 (d)	76.2 (d)	76.2 (d)		77.1 (d)	77.0 (d)
6''	67.1 (t)	67.2 (t)	67.3 (t)		61.3 (t)	61.3 (t)
rha						
1'''	101.1 (d)	101.2 (d)	101.1 (d)	102.6 (d)		
2'''	70.5 (d)	70.6 (d)	70.7 (d)	71.4 (d)		
3'''	70.8 (d)	70.8 (d)	70.9 (d)	72.1 (d)		
4'''	72.0 (d)	72.2 (d)	72.2 (d)	72.9 (d)		
5'''	68.5 (d)	68.6 (d)	68.6 (d)	71.3 (d)		
6'''	17.9 (q)	18.1 (q)	18.1 (q)	17.7 (q)		

\* : δ in acetone-*d*<sub>6</sub>

respectively.

Structure identification

Compound 1 C<sub>29</sub> H<sub>34</sub> O<sub>16</sub>, pale yellow amorphous powder (MeOH), with a positive reaction to Molish and Mg-HCl tests. mp 156-158℃; UV (MeOH) λ<sub>max</sub> nm: 253, 353; IR (KBr) cm<sup>-1</sup>: 3380 (OH), 1650 (C=O), 1607, 1502 (C=C); ESI-MS *m/z* 639 [M + H]<sup>+</sup>, 493[M + H - rha]<sup>+</sup>, 331[M + H - rha - glc]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 0.97 (3H, d, *J* = 6.4 Hz, Me of rhamnose), 3.85 (3H, s, MeO-7), 3.87 (3H, s, MeO-3'), 4.41 (1H, brs, H-1''' of rhamnose), 5.44 (1H, d, *J* = 7.6 Hz, H-1'' of glucose), 6.37 (1H, d, *J* = 2.0 Hz, H-6), 6.71 (1H, d, *J* = 2.0 Hz, H-8), 6.92 (1H, d, *J* = 8.4 Hz, H-5'), 7.57 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 7.87 (1H, d, *J* = 2.0 Hz, H-2'), 9.71 (1H, brs, 4'-OH), 12.56 (1H, brs, 5-OH). <sup>13</sup>C NMR data see Table 1. Above data were in accord with those of rhamnazin-3-O-β-D-rutinoside<sup>[5]</sup>. Therefore, compound 1 was identified as rhamnazin-3-O-β-D-rutinoside.

Compound **2** C<sub>28</sub>H<sub>32</sub>O<sub>6</sub>, pale yellow amorphous powder (MeOH), mp 202-204°C; UV (MeOH) λ<sub>max</sub> nm: 255, 355; IR (KBr) cm<sup>-1</sup>: 3375 (OH), 1630 (C=O), 1605, 1500 (C=C); ESI-MS *m/z* 625 [M + H]<sup>+</sup>, 479 [M + H - rha]<sup>+</sup>, 317 [M + H - glc - rha]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 0.99 (3H, d, *J* = 6.4 Hz, Me of rhamnose), 3.85 (3H, s, MeO-7), 4.39 (1H, brs, H-1'' of rhamnose), 5.29 (1H, d, *J* = 7.6 Hz, H-1'' of glucose), 6.34 (1H, d, *J* = 2.0 Hz, H-6), 6.67 (1H, d, *J* = 2.0 Hz, H-8), 6.74 (1H, d, *J* = 8.8 Hz, H-5'), 7.56 (1H, d, *J* = 2.0 Hz, H-2'), 7.59 (1H, dd, *J* = 8.8, 2.0 Hz, H-6'), 12.89 (1H, brs, 5-OH). <sup>13</sup>C NMR data see Table 1. Above data were in agreement with those of rhamnetin-3-O-β-D-rutinoside<sup>[6]</sup>.

Compound **3** C<sub>27</sub>H<sub>30</sub>O<sub>6</sub>, yellow needle crystals (MeOH), mp 176-178°C; UV (MeOH) λ<sub>max</sub> nm: 258, 357; IR (KBr) cm<sup>-1</sup>: 3420 (OH), 1656 (C=O), 1602, 1502 (C=C); ESI-MS *m/z*: 611 [M + H]<sup>+</sup>, 465 [M + H - rha]<sup>+</sup>, 303 [M + H - glc - rha]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 1.00 (3H, d, *J* = 6.0 Hz, Me of rhamnose), 4.40 (1H, d, *J* = 1.6 Hz, H-1''' of rhamnose), 5.32 (1H, d, *J* = 7.2 Hz, H-1'' of glucose), 6.15 (1H, d, *J* = 2.0 Hz, H-6), 6.35 (1H, d, *J* = 2.0 Hz, H-8), 6.83 (1H, d, *J* = 8.4 Hz, H-5'), 7.52 (1H, d, *J* = 2.0 Hz, H-2'), 7.54 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 12.89 (1H, brs, 5-OH). <sup>13</sup>C NMR data see Table 1. Above data were consistent with those of quercetin-3-O-β-D-rutinoside (rutin)<sup>[7]</sup>.

Compound **4** C<sub>21</sub>H<sub>20</sub>O<sub>10</sub>, yellow needle crystals (MeOH), mp 175-178°C; UV (MeOH) λ<sub>max</sub> nm: 266, 344; IR (KBr) cm<sup>-1</sup>: 3300 (OH), 1660 (C=O), 1610, 1500 (C=C); ESI-MS *m/z*: 433 [M + H]<sup>+</sup>, 287 [M + H - rha]<sup>+</sup>; <sup>1</sup>H NMR (Acetone-*d*<sub>6</sub>) δ: 0.86 (3H, d, *J* = 6.0 Hz, Me of rhamnose), 5.50 (1H, d, *J* = 1.6 Hz, H-1'' of rhamnose), 6.23 (1H, d, *J* = 2.0 Hz, H-6), 6.43 (1H, d, *J* = 2.0 Hz, H-8), 6.98 (2H, d, *J* = 8.8 Hz, H-3', 5'), 7.81 (2H, d, *J* = 8.8 Hz, H-2', 6'), 9.29 (1H, brs, 7-OH), 9.91 (1H, brs, 4'-OH), 12.68 (1H, brs, 5-OH). <sup>13</sup>C NMR data see Table 1. Above data were in accord with those of kaempferol-3-O-α-L-rhamnopyranoside (afzelin)<sup>[8]</sup>.

Compound **5** C<sub>16</sub>H<sub>28</sub>O<sub>7</sub>, colourless oil; [α]<sub>D</sub><sup>28</sup> 3/ 16.3° (c 0.50, MeOH); ESI-MS *m/z*: 355 [M + Na]<sup>+</sup>, 333 [M + H]<sup>+</sup>, 315 [M + H - H<sub>2</sub>O]<sup>+</sup>, 153 [315 - glc]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 1.15 (3H, s, Me-9), 1.43 (2H, t, *J* = 8.0 Hz, H-4), 1.59 (3H, s, Me-10), 1.99 (2H, m, H-5), 3.88 (1H, d, *J* = 12.0 Hz, H-8a), 4.07 (1H, d, *J* = 12.0 Hz, H-8b), 4.08 (1H, d, *J* = 8.0 Hz, H-1'' of glucose), 4.95 (1H, brd, *J* = 10.8 Hz, H-1a), 5.14 (1H, brd, *J* = 17.2 Hz, H-1b), 5.40 (1H, t, *J* = 6.0 Hz, H-6), 5.86 (1H, dd, *J* = 17.2, 10.8 Hz, H-2). <sup>13</sup>C NMR data see Table

1. Above data were in conformity with those of betulabuside A<sup>[9]</sup>.

Compound **6** C<sub>16</sub>H<sub>30</sub>O<sub>7</sub>, colourless oil; [α]<sub>D</sub><sup>28</sup> 3/ 50.3° (c 0.50, MeOH); ESI-MS *m/z*: 357 [M + Na]<sup>+</sup>, 335 [M + H]<sup>+</sup>, 317 [M + H - H<sub>2</sub>O]<sup>+</sup>, 155 [317 - glc]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 0.87 (3H, d, *J* = 6.8 Hz, Me-10), 1.03 (1H, m, H-6a), 1.13 (3H, s, Me-9), 1.15 (1H, m, H-6b), 1.35 (2H, m, H-4), 1.36 (2H, m, H-5), 1.64 (1H, m, H-7), 3.45 (1H, dd, *J* = 12.0, 6.0 Hz, H-8a), 3.66 (1H, dd, *J* = 12.0, 6.0 Hz, H-8b), 4.08 (1H, d, *J* = 7.6 Hz, H-1'' of glucose), 4.92 (1H, brd, *J* = 10.8 Hz, H-1a), 5.11 (1H, brd, *J* = 17.2 Hz, H-1b), 5.85 (1H, dd, *J* = 17.2, 10.8 Hz, H-2). <sup>13</sup>C NMR data see Table 1. Above data were in agreement with those of 3,7-dimethyloct-1-en-3,8-diol-8-O-β-D-glucopyranoside<sup>[10]</sup>.

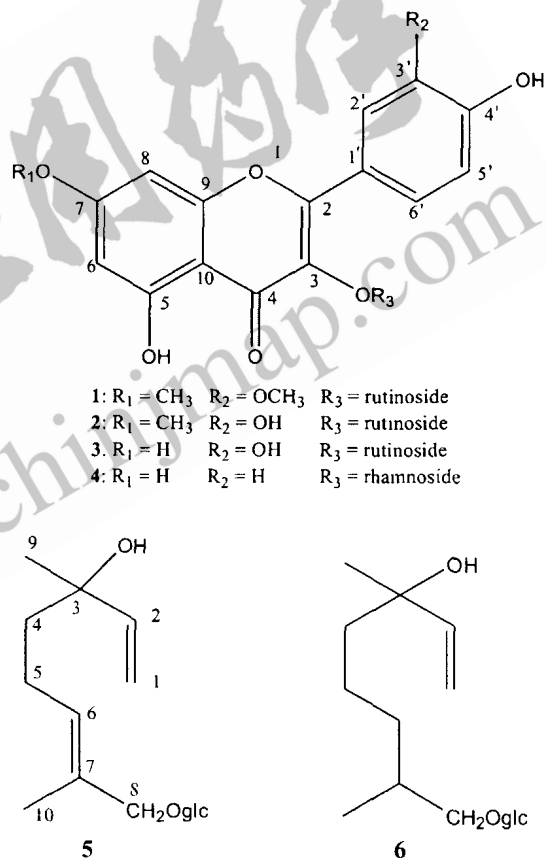


Fig 1 Structures of compounds 1-6

图 1 化合物 1~6 的化学结构

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