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藏药臭蚤草的一个新的苯丙素苷类成分

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摘 要:为了研究藏药臭蚤草的活性成分,我们利用各种柱色谱技术,从藏药臭蚤草甲醇提取物的乙酸乙酯萃取相分离到 4个化合物,通过 1D、2D NMR、MS和 HRMS等试验,鉴定为 2,4-dihydroxy-6-methyl-ethanone-4-O-β-*D*-Glc(**1**),4-(3′-hydroxypropyl)-2,6-dimethoxyphenol-3′-O-β-*D*-glcoside(**2**),4-allyl-2-methoxyphenol-1-O-β-*D*-glcoside(**3**),2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6′-O-Ac)-α-*L*-Rha-(1→2)-β-*D*-Glc(**4**),其中化合物 1～3为苯丙素苷类化合物,化合物 4为蒽醌苷。化合物 1～4都是首次从该属植物中分离得到,化合物 1为新的苯丙素苷类化合物。

关键词:藏药;臭蚤草;苯丙素苷;蒽醌苷

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A New Phenylpropanoid Glycoside from Tibetan Folk Drug *Pulicaria insignis*

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Abstract: In order to investigate the bioactivity constituents of Tibetan folk drug *Pulicaria insignis*, four compounds were isolated with chromatography techniques. Structures were elucidated to be 2,4-dihydroxy-6-methyl-ethanone-4-O-β-*D*-Glc(**1**),4-(3′-hydroxypropyl)-2,6-dimethoxyphenol-3′-O-β-*D*-glcoside(**2**),4-allyl-2-methoxyphenol-1-O-β-*D*-glcoside(**3**),2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6′-O-Ac)-α-*L*-Rha-(1→2)-β-*D*-Glc(**4**) on the basis of spectroscopic analysis including 1D,2D-NMR,MS and HRMS. Four compounds were isolated from the genus *Pulicaria* for the first time. 2,4-Dihydroxy-6-methyl-ethanone-4-O-β-*D*-Glc(**1**) is a new phenylpropanoid glycoside.

Key words: Tibetan folk drug; *Pulicaria insignis*; phenylpropanoid glycoside; anthraquinone glycoside

Introduction

Pulicaria insignis of the family Asteraceae (Compositae) grows at alpine ultimate natural environment more than 4000 m above sea level. This species are used traditionally in the treatments of fever, pain and cough[1]. There is no report on its chemical constitutes study in literatures.

Previous study of chemical constituents for genus *Pulicaria* were focused on diquiterpenoids[2-6] and sesquiterpenoids[7-11]. However, glycosides from this genus were hardly reported. To find more bioactive compounds, constituents investigation for *P. insignis* was performed. Herein, we report the isolation and identification for one new phenylpropanoid glycoside, 2,4-dihydroxy-6-methyl-ethanone-4-O-β-*D*-Glc(**1**), which is different from 2,4-dihydroxy-6-methyl-ethanone-4-O-α-*L*-glcoside(acetophenone)[12], and three known compounds 4-(3′-hydroxypropyl)-2,6-dimethoxyphenol-3′-O-β-*D*-glcoside(**2**)[13], 4-allyl-2-methoxyphenol-1-O-β-*D*-glcoside(**3**)[14-16] and an anthraquinone glyco side 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-(6′-O-Ac)-α-*L*-Rha-(1→2)-β-*D*-Glc(**4**)[17] (Fig 1).

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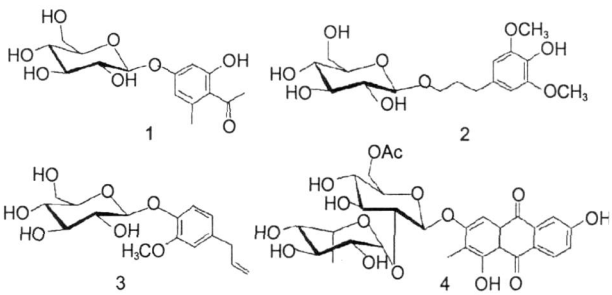

Fig. 1 Structures of compounds 1-4

Experimental Section

Optical rotation determinations were carried on an OA AA-55 polarimeter. IR spectra were recorded by a Bio-Rad FTS-135 spectrometer with KBr pellets. 1D-and 2D-NMR spectra were recorded on Burker AM-400 and DRX-500 instruments with TMS as an internal standard. The MS data were recorded by a VG Auto Spec-3000 Spectrometer. Column chromatography was performed on Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co. Ltd.), Chromatorex ODS (100-200 μm, YMC Co. Ltd.) and silica gel (200-300 mesh, Qingdao Marine Chemistry Inc.). Thin-layer chromatography (TLC) was carried out on silica gel G pre-coated plates (Qingdao Marine Chemistry Inc.) and compounds were detected by baptizing TLC in sulfuric acid reagents (5% in ethanol) followed by heating.

Plant material

The herb of *P. insignis* was collected in Lhasa area of Tibet, China, in October 2007, and identified by Professor Si-Ping Jiang, Plateau Institute of Biology Lhasa Tibet of P. R. China where the specimen was deposited.

Extraction and isolation

Row herb powder of *P. insignis* (90 kg) was extracted with methanol and the extraction (2.5 kg) was obtained and extracted with petroleum ether to remove oils. It was then extracted with ethyl ester, the extracted fraction was subjected to column chromatography over silica gel (eluded with $CHCl_3$/MeOH 1:0-1:2), and the $CHCl_3$/MeOH (1:2) was then subjected to column chromatography again over ODS (eluded with H_2O/MeOH 1:9 to 1:0), and Sephadex LH-20 (eluted with $CHCl_3$/MeOH 1:1 or 0:1) repeatedly to yield compounds **1** (5 mg), **2** (3 mg), **3** (4 mg) and **4** (41 mg).

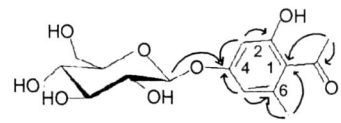

Fig. 2 Key HMBC correlations of compound 1

Results and Discussion

Compound **1** was obtained as a yellow oil with optical rotation $[\alpha]_D^{21}$ -46.8 °(*c* 0.00235, MeOH). Its molecular formula was deducted as $C_{15}H_{20}O_8$ by HR-ESHMS at *m/z* 289.1418 ([$C_{15}H_{22}O_4$ + Na]$^+$ Calcd. *m/z*: 289.1415). IR absorption at 3338 cm^{-1} showed the existence of hydroxyl group. Carbonyl group (1620 cm^{-1}) and aromatic ring (1490, 1430, 1420 cm^{-1}) were found. In ^{1}H NMR spectrum of **1**, two single signals (δ 6.46 and 6.45) showed that there were four substituents on phenyl ring, the two protons must be isolated by the four substituents. Moreover, the shifts of proton at 4.90 (m, 1H) [4.99 (d, 1H, *J* = 7.7 Hz) in $(CD_3)_2CO$] and of carbon at 101.6 (d), 74.7 (d), 77.9 (d), 71.2 (d), 78.2 (d) and 62.4 (t) showed the presence of a O-β-glucosyl moiety [13-15]. One methyl (δ 2.32, s, 3H) and one acetyl group (δ 2.54, s, 3H; δ 32.7 (q) and 207.1 (s)) were found. Two bearing-oxygen aromatic carbons (δ 161.0 (s) and 161.5 (s)) were observed. In HMBC, the relationship of methyl of acetyl group δ 2.54 (s, 3H) with the aromatic carbon [C-1, δ 122.2 (s)] was found and this hinted acetyl connected to aromatic C-1. Methyl group δ 2.32 (s, 3H) had correlations to aromatic carbon at δ 122.2 (s), 111.9 (d), 140.9 (s) in HMBC, this clearly exhibited this methyl located at C-6 (Fig. 2). The correlation of aromatic protons δ 6.46 (s, 1H), 6.45 (s, 1H) and H-1′ of glucose δ 4.90 (m, 1H) [4.99 (d, 1H, *J* = 7.7 Hz) in $(CD_3)_2CO$] with aromatic carbon δ 161.5 (s) revealed that glucosyl located at C-4 with β-connection. One proton connected on C-3 δ 102.6 (d). A hydroxyl group was linked at C-2 δ 161.0 (s). Structure of **1** was assigned as 2, 4-dihydroxy-6-methyl-ethanone-4-O-β-*D*-Glucoside (Fig. 2).

The NMR data for other three compounds are summarized below:

Table 1 ^{1}H NMR (400 MHz, CD_3OD, δ, J_{Hz}) and ^{13}C NMR (100 MHz, CD_3OD, δ) of compound 1

No.	δ_H	δ_C	No.	δ_H	δ_C
1	–	122.2 (s)	Glc		
2	–	161.0 (s)	1′	4.90 (m, 1H)	101.6 (d)
3	6.46 (s, 1H)	102.6 (d)	2′	3.44 (m, 1H)	74.7 (d)
4		161.5 (s)	3′	3.43 (m, 1H)	77.9 (d)
5	6.45 (s, 1H)	111.9 (d)	4′	3.38 (m, 1H)	71.2 (d)
6	-	140.9 (s)	5′	3.47 (m, 1H)	78.2 (d)
C=O	-	207.1 (s)	6′	3.90 (dd, 1H, *J* = 1.7, 13.7 Hz) 3.70 (dd, 1H, *J* = 5.5, 12.0 Hz)	62.4 (t)
CH_3 (C=O)	2.54 (s, 3H)	32.7 (q)			
CH_3	2.32 (s, 3H)	21.9 (q)			
-OH	4.66 (s, br)				

2, 4-dihydroxy-6-methyl-ethanone-4-O-β-D-Glucoside (1) $C_{15}H_{20}O_8$ light yellow oil, $[\alpha]_D^{21}$ -46.8 (*c* 0.00235, MeOH); IRυ_{max}^{KBr} cm^{-1}: 3338, 2930, 2886, 1620, 1490, 1430, 1420, 1377, 1359, 1262, 1222, 1184, 1074, 885, 849, 833, 667; ESI-MS (pos.) *m/z*: 329 $[M+1]^+$ (2.5), 351 $[M+Na]^+$ (10.0), 567 $[M+K]^+$ (2.0); HR ESI-MS (pos.) 351.1050 ($[M+Na]^+$, $[C_{15}H_{20}O_8+Na]^+$ Calcd. 351.1055), For ^{1}H and ^{13}C NMR spectrum data in CD_3OD see the Table 1. ^{1}H NMR ($(CD_3)_2CO$, 500 MHz) δ: 12.1 (s, br), 6.44 (s, 1H), 6.43 (s, 1H), 5.99 (d, 1H, *J* = 7.7 Hz), 4.68 (s, br), 4.45 (s, br), 4.38 (s, br), 3.84 (m, 2H), 3.68 (m, 1H), 3.53 (m, 1H), 3.48 (m, 2H), 2.58 (s, 3H), 2.47 (s, 3H).

4-(3′-hydroxypropyl)-2, 6-Dimethoxyphenol-3′-O-β-D-Glc (2) Colourless oil, $[\alpha]_D^{21}$ -20.7 (*c* 0.000159, MeOH), $C_{17}H_{26}O_9$, ESI-MS (neg.) *m/z*: 409 $[M+Cl]^-$ (110). ^{1}H NMR (CD_3OD, 400 MHz) δ: 6.54 (s, 2H), 4.79 (d, 1H, *J* = 7.2Hz), 3.85 (s, 1H), 3.83 (s, 6H), 3.77 (dd, 1H, *J* = 7.7, 11.9 Hz), 3.68 (dd, 1H, *J* = 5.1, 6.4 Hz), 3.57 (t, 2H, *J* = 12.8 Hz), 3.46 (m, 3H), 3.20 (s, br, 1H), 2.63 (t, 2H, *J* = 15.4 Hz), 1.82 (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 154.1 (s), 140.4 (s), 135.0 (s), 107.4 (d), 105.6 (d), 78.3 (d), 77.8 (d), 75.7 (d), 71.3 (d), 62.6 (t), 62.1 (t), 56.9 (q), 35.4 (t), 33.4 (t).

4-allyl-2-methoxyphenoxy-4-β-D-Glc (3) Colourless oil, $[\alpha]_D^{21}$ -40.4 (*c* 0.00125, MeOH), $C_{16}H_{22}O_7$. ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.07 (d, 1H, *J* = 8.2Hz), 6.82 (s, 1H), 6.71 (d, 1H, *J* = 8.2Hz), 5.95 (m, 1H), 5.05 (s, 1H), 5.02 (d, 1H, *J* = 9.6 Hz), 4.85 (d, 1H, *J* = 7.32Hz), 3.87 (s, 1H), 3.84 (d, 3H, *J* = 17.8 Hz), 3.45 (dd, 1H, *J* = 6.0, 7.4 Hz), 3.33 (m, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 150.7 (s), 146.3 (s), 139.0 (d), 136.4 (s), 122.1 (d), 118.2 (d), 115.8 (t), 114.1 (d), 103.0 (d), 78.2 (d), 77.8 (d), 74.9 (d), 71.3 (d), 62.5 (t), 56.6 (q), 40.8 (t).

2-methyl-1, 3, 6-trihydroxy-9, 10-anthraquinone-3-O-(6′′-O-Ac)-α-L-Rha-(1→2)-β-D-Glc (4) Orange needle crystal, $[\alpha]_D^{21}$ -18.5 (*c* 0.0020, MeOH), $C_{29}H_{32}O_{15}$, ESI-MS (neg.) *m/z*: 619 $[M-1]^-$ (16.0), 655 $[M+Cl]^-$ (1.0), HR ESI-MS (neg.) 619.1651 ($[M-1]^-$, $[C_{29}H_{32}O_{15}-H]^-$ Calcd. *m/z*: 619.1662). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.23 (s, br, 1H), 11.10 (s, br, 1H), 8.09 (d, 1H, *J* = 6.84 Hz), 7.47 (d, 1H, *J* = 1.92 Hz), 7.40 (s, 1H), 7.23 (dd, 1H, *J* = 2.0, 6.9 Hz), 5.46 (m, 3H), 5.27 (s, 1H), 4.68 (d, 1H, *J* = 3.36 Hz), 4.48 (d, 1H, *J* = 4.7 Hz), 4.33 (s, 1H), 4.31 (s, 1H), 3.99 (dd, 1H, *J* = 6.4, 9.6 Hz), 3.77 (dd, 1H, *J* = 7.6, 7.6 Hz), 3.69 (s, 1H), 3.63 (m, 3H), 3.20 (m, 2H), 2.14 (s, 3H), 1.92 (s, 3H), 1.07 (d, 3H, *J* = 4.92 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 186.4 (s), 181.7 (s), 170.3 (s), 163.6 (s), 161.3 (s), 160.0 (s), 135.3 (s), 131.9 (s), 129.7 (d), 124.5 (s), 121.5 (d), 120.5 (s), 112.5 (d), 110.6 (s), 105.3 (d), 100, 2 (d), 97.2 (d), 77.0 (s), 76.2 (s), 74.0 (d), 71.9 (d), 70.4 (d), 70.3 (d), 70.0 (d), 68.5 (d), 63.3 (t), 20.4

(q), 18.1 (q), 8.72 (q).

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