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黑蕊虎耳草中岩白菜素没食子酸酯类及其对丙型肝炎丝氨酸蛋白酶的抑制作用*

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摘要: 研究了黑蕊虎耳草 (*Saxifraga melanocentra*) 中岩白菜素衍生物的化学和生物活性, 从其地上部分分离纯化得到一个新的岩白菜素没食子酸酯, 主要通过 1 维和 2 维核磁共振波谱鉴定结构为 11-氧-(4-氧甲基没食子酰) 岩白菜素 [11-*O*-(4-*O*-methylgalloyl) bergenin (**1**)], 该化合物对丙型肝炎丝氨酸蛋白酶 (HCV NS3 serine protease) 具有抑制活性, 其 IC_{50} 为 0.32 mg/mL。

关键词: 抗丙型肝炎; 黑蕊虎耳草; 11-氧-(4-氧甲基没食子酰) 岩白菜素; IC_{50}
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Gallic Acid Esters of Bergenin from *Saxifraga melanocentra* (Saxifragaceae) and Their Inhibition Against HCV NS3 Protease

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Abstract: To study the chemical and bioactive characteristics of bergenin derivatives which were purified from *Saxifraga melanocentra* Franch., a new gallic acid ester of bergenin, 11-*O*-(4-*O*-methylgalloyl) bergenin (**1**) was isolated from the aerial parts of *Saxifraga melanocentra* Franch., and its structure was established mainly on the basis of 1D and 2D NMR spectroscopic analysis. It showed weak inhibitory activity against HCV NS3 serine protease with IC_{50} of 0.32 mg/ml.
Key words: HCV NS3 serine protease; *Saxifraga melanocentra*; 11-*O*-(4-*O*-methylgalloyl)-bergenin; IC_{50}

Bergenin (Chinese named yan-baicai-su), a famous antitussive agent which has been used clinically for many years, is a major constituent from the genus of *Bergenia* and *Saxifraga* (Saxifragaceae) and the other plant species. It showed a variety of biological activities, including antioxidant, hepatoprotective (Kim *et al*. 2000), antiarrhythmic (Pu *et al*. 2002), anti-ulcer (Goel *et al*. 1997) and anti-HIV (Piacente *et al*. 1996). In the course of our screening natural products as Hepatis C virus (HCV) inhibitors, we studied the active constituents of *Saxifraga melanocentra* Franch previously (Zuo *et al*. 2005a). Further investigation of its active constitution led us to isolate a new bergenin derivative, 11-*O*-(4-*O*-methylgalloyl) bergenin **1**, and four known ones, i.e. 11-*O*-(3,4-di-*O*-methylgalloyl) bergenin **2** (Jia *et al*. 1995), 11-*O*-

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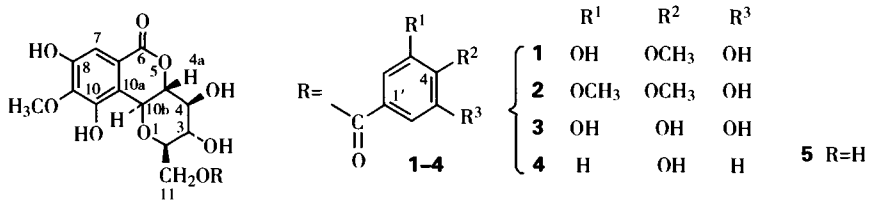

Fig. 1 Structures of compounds **1–5**

galloylbergenin **3** (Yoshida *et al.* 1982), 11-*O*-(4-hydroxy benzoyl) bergenin **4** (Fuji *et al.* 1996) and bergenin **5** (Taneyama *et al.* 1983). The present paper describes the structural elucidation of the new gallic acid ester of bergenin (Fig. 1). Compounds **1 – 5** showed weak to moderate inhibitory activities against HCV NS3 serine protease (Table 1).

Compound **1** was obtained as colorless needles, whose molecular formula $C_{22}H_{22}O_{13}$ was established by HRESIMS spectrum (*m/z* 493.1057 [M-1]$^-$, calcd. for $C_{22}H_{21}O_{13}$ 493.1060). The IR absorptions at 3390, 3250, 1726, 1704, 1608, 1464 cm^{-1} suggested the presence of hydroxyl and carbonyl groups. Its ^{13}C NMR spectra exhibited characteristics of a bergenin moiety, including a lactone group (δ 163.7), two phenolic hydroxyl groups (δ 151.7 and 148.9) and a phenoxy-

Table 1 Inhibitory activities of compounds **1–5** against HCV NS3 serine protease[a]

compounds	IC_{50} (mg/ml)
1	0.32 ± 0.08
2	> 1.00
3	0.07 ± 0.02
4	> 1.00
5	0.56 ± 0.13

[a] The results are the mean values of triplicate tests.

methyl group (δ 141.3, 60.6), five tertiary oxygenated and a second oxygenated linkages, which were closely similar to those of compound **5**. Its ^{1}H NMR spectrum showed two methoxyl singlets at δ 3.88 (s, Me) and 3.89 (s, Me), whose attached carbons were overlapped and exhibited strongly at δ 60.6 in the ^{13}C NMR spectrum. This suggested the two methoxyl groups were in the similar chemical situation. There were three aromatic singlets at δ 7.09, 7.17 and 7.17, of which the latter two were also overlapped to double approximately their height in comparison with the former singlet. The same phenomenon was further observed by their corresponding signals at δ 110.3, 110.0 and 110.0 in the ^{13}C NMR spectrum, of which two carbonyl signals at δ 163.7 and 166.5 were designated to C–6 and C–1′, respectively. The presence of a methylated galloyl moiety, to which the C–11 methylene was attached by an ester linkage, was suggested by the HMBC correlation of H–11 with carbon in the ester, and a methoxyl group was attached to C–4′ of the galloyl moiety, also suggested by the HMBC correlation of them. Therefore, the structure of Compound **1** was elucidated as 11-*O*-(4-*O*-methylgalloyl) bergenin. All the signals of protons and carbons of **1** were assigned unambiguously through analysis of the spectra of DEPT, HSQC and HMBC (Fig. 2), and compared with the corresponding data of compound **3**. Identification of compounds **2–5** were performed by analysis of their spectra of MS, IR, NMR and compared with literatures and an authentic sample of bergenin (**5**).

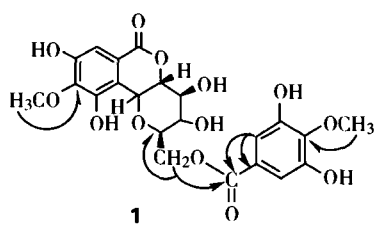

Fig. 2 Key HMBC correlations for **1**

Experimental

Melting points were determined using a Kofler micro-melting point apparatus and are uncorrected. Optical rotations were determined on a Horiba SEPA-300 polarimeter. ^{1}H and ^{13}C NMR spectra were determined on a Bruker ARX400 spectrometer at 400 and 100 MHz, respectively, in CD_3OD, with $SiMe_4$ as internal standard; chemical shifts are given as (ppm) values. IR spectra were recorded on a Nicolet Magana-IR750. Electrospray ionization and time of flight Mass spectra (ESI-TOF-MS) were taken on a Mariner Biospectrometry workstation. TLC plates were made with polyamide film (China), and spots were detected by ultraviolet (UV) and by spraying with $FeCl_3$ (5% in ethanol) reagent. Polyamide (100 – 200 mesh, China) and Sephadex. LH-20 (Amersham Pharmacia Biotech) was used for column chromatography (C. C). The organic solvents used were of analytical grade. Solvents in the extracts were evaporated under re-

duced pressure at below 40℃.

The aerial parts of *S. melanocentra* were bought from the Qinghai Institute of Tibetan Medicines (Qinghai Province, China) in October, 2002, where a voucher specimen has been deposited.

Part of the experimental materials and methods have been reported previously (Zuo *et al*. 2005b). The powdered aerial part of *S. melanocentra* (1 kg) were extracted with ethanol (80%, 4 L×3) at room temperature and concentrated to dryness in vacuum to give a crude extract (205 g). The extract was partitioned between H_2O (600 mL) and petroleum ether (60–90℃), chloroform, ethyl acetate and *n*-butanol (250 mL×3 each) successively. The ethyl acetate layer part (20 g) having the most potent inhibitory activity for HCV NS3 serine protease by ca 80% at 100 mg/mL was fractionated over silica gel C. C. with solvent systems as ethyl acetate-methanol-H_2O (10:1:0–7:1.5:1) to give four active fractions (Frs-1-4). The more active fraction (Fr-3, 4.5 g) was further isolated by polyamide C. C. (ethyl acetate-methanol-H_2O (120:13:5–77:13:10)) to give compounds 1–5 (50 mg, 20 mg, 16 mg, 22 mg, 3.4 g), respectively (Fig. 1).

Compound **1**, colorless needles (MeOH), mp: 142–144℃; $[\alpha]20_D$: +39.3° (*c*, 0.5, EtOH); EIMS *m/z* (%): 494 (3), 328 (4), 208 (14), 184 (12), 167 (22), 138 (63), 121 (100), 93 (26); HRESIMS: (*m/z* 493.1057 $[M-1]^-$, calcd. for $C_{22}H_{21}O_{13}$ 493.1060). IR ν_{max}^{KBr} cm^{-1}: 3390, 3250 (OH), 2852 (CH_2), 1726, 1704 (C=O), 1608, 1527 (aromatic), 1464, 1349, 1236 (aromatic C-O), 864 (C-H); ^{1}H-NMR (400 MHz, CD_3COCD_3, (ppm, *J* Hz): δ 7.17 (s, 2H, H-2′, 6′), 7.09 (s, 1H, H-7), 5.17 (d, 1H, *J* = 10.4, H-10b), 4.94 (d, 1H, *J* = 2.0, H-11α), 4.45 (d, 1H, *J* = 5.6 H-11β), 4.16 (m, 1H, H-4a), 4.13 (m, 1H, H-2), 3.96 (m, 1H, H-4), 3.89 (s, 3H, C-9-OC$\underline{H}_3$), 3.88 (s, 3H, C-4′-OC$\underline{H}_3$), 3.72 (m, 1H, H-3); ^{13}C-NMR (100 MHz, CD_3COCD_3, δ ppm): 166.5 (s, C$\underline{O}$O-methylgalloyl), 163.7 (s, C-6), 151.7 (s, C-8), 151.3 (s, C-3′, 5′), 148.9 (s, C-10), 141.3 (s, C-9), 140.7 (s, C-4′), 126.0 (s, C-1′), 119.5 (s, C-6a), 116.7 (s, C-10a), 110.3 (d, C-7), 110.0 (d, C-2′, 6′), 80.7 (d, C-4a), 80.1 (d, C-2), 75.3 (d, C-4), 74.1 (d, C-10b), 71.5 (d, C-3), 64.3 (t, C-11), 60.6 (q, C-9-O$\underline{C}H_3$, C-4′-O$\underline{C}H_3$).

Inhibitory activities of compounds **1–5** against HCV NS3 serine protease were determined in our laboratory by ELISA (Table 1). A peptide substrate with an acetyl group at N-terminus and a biotin at C-terminus was hydrolyzed by NS3 protease into product with a free amino moiety at N-terminus. The product was immobilized and the free amino moiety was analyzed (Takeshita *et al*. 1997; Zuo *et al*. 2005a).

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