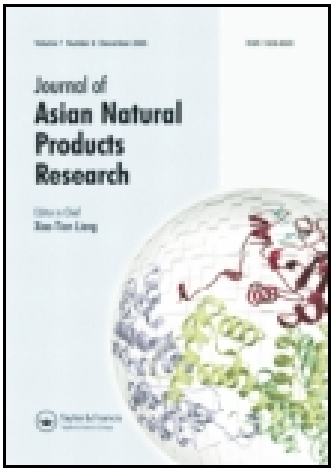


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# Grasshopper ketone 3-O-primveroside from Sinocrassula indica

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# Grasshopper ketone 3-*O*-primveroside from *Sinocrassula indica*

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A new megastigmane glycoside, grasshopper ketone 3-*O*-primveroside (**1**), was isolated from the methanolic extract of the whole herbs of *Sinocrassula indica* (Crassulaceae). Its structure was elucidated on the basis of spectral and chemical evidence.

**Keywords:** *Sinocrassula indica*; Crassulaceae; grasshopper ketone; grasshopper ketone 3-*O*-primveroside



## 1. Introduction

*Sinocrassula indica* (Decaisne) A. Berger (Crassulaceae) is biennial herbs distributed in south and southwest China, Bhutan, India, Nepal, Pakistan, and Sikkim [1]. It has been used as a folk medicine for the treatment of hepatitis and otitis medium and also as vegetable and herbal tea [2]. Our previous phytochemical studies on the whole herbs of this species disclosed 35 flavonoids and two known megastigmane glycosides [3–6]. Recently, we have re-examined the spectral data of a compound previously isolated but its structure remains uncharacterized and it was found to be a megastigmane, glycoside possessing a rare grasshopper ketone aglycone. Herein, we report the isolation and structural elucidation of this new compound.

## 2. Results and discussion

The methanolic extract of *S. indica* was dissolved in water and sequentially fractionated with EtOAc and *n*-BuOH. The resultant *n*-BuOH-soluble fraction was subjected to silica gel and ODS column chromatography, respectively, and finally subjected to preparative HPLC to yield compound **1**.

Compound **1**, obtained as white amorphous powder, was deduced the molecular formula $C_{24}H_{38}O_{12}$ from its quasi-molecular ion peaks at *m/z* 541 $[M + Na]^+$ and 517 $[M - H]^-$ in the FAB-MS as well as *m/z* 541.2261 $[M + Na]^+$ in the HR-FAB-MS. The IR spectrum showed absorption bands for hydroxyl (3450 $cm^{-1}$), allenic group (1945 $cm^{-1}$), and conjugated carbonyl (1673 $cm^{-1}$). The $^1H$ NMR spectrum (Table 1) exhibited signals readily recognized in $C_5D_5N$ for an olefinic proton at $\delta$ 5.90 (s, H-8), two anomeric protons with β-configuration at $\delta$ 5.05 (d, $J = 7.9$ Hz, H-1′) and 4.99 (d, $J = 6.7$ Hz, H-1″), a carbinol proton at $\delta$ 4.98 (m, H-3) and four tertiary methyls at $\delta$ 2.20 ($CH_3$-10), 1.61 ($CH_3$-11), 1.52 ($CH_3$-13), and 1.14 ($CH_3$-12). The $^{13}C$ NMR and DEPT spectra (Table 1) exhibited 24 carbon signals, in $C_5D_5N$ which consisted of 4 methyls, 4 methylenes including 2 oxygenated methylenes at $\delta$

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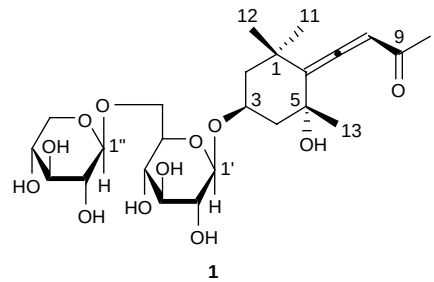
Table 1. $^{1}H$ and $^{13}C$ NMR (DEPT) spectral data of compound **1**.

| | $C_5D_5N$ | | $CD_3OD$ | |
|---|---|---|---|---|
| H/C | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ (DEPT) | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ (DEPT) |
| 1 | | 36.3 (C) | | 37.0 (C) |
| 2α | 2.86 (dd, 12.0, 3.0) | 46.8 ($CH_2$) | 2.86 (dd, 11.3, 3.0) | 46.6 ($CH_2$) |
| 2β | 1.65 (dd, 12.0, 12.5) | | 1.45 (dd, 11.3, 13.1) | |
| 3 | 4.98 (m) | 71.7 (CH) | 4.33 (m) | 72.7 (CH) |
| 4α | 2.48 (dd, 12.2, 3.4) | 47.9 ($CH_2$) | 2.08 (dd, 11.3, 3.4) | 48.1 ($CH_2$) |
| 4β | 1.70 (dd, 12.2, 12.5) | | 1.46 (dd, 11.3, 13.1) | |
| 5 | | 71.3 (C) | | 72.4 (C) |
| 6 | | 119.8 (C) | | 120.1 (C) |
| 7 | | 197.8 (C) | | 200.9 (C) |
| 8 | 5.90 (s) | 100.5 (CH) | 5.83 (s) | 101.2 (CH) |
| 9 | | 209.6 (C) | | 211.5 (C) |
| 10 | 2.20 (3H, s) | 26.4 ($CH_3$) | 2.19 (3H, s) | 26.6 ($CH_3$) |
| 11 | 1.61 (3H, s) | 29.3 ($CH_3$) | 1.39 (3H, s) | 29.5 ($CH_3$) |
| 12 | 1.14 (3H, s) | 32.0 ($CH_3$) | 1.16 (3H, s) | 32.3 ($CH_3$) |
| 13 | 1.52 (3H, s) | 31.1 ($CH_3$) | 1.39 (3H, s) | 30.9 ($CH_3$) |
| 1′ | 5.05 (d, 7.9) | 102.7 (CH) | 4.45 (d, 8.0) | 102.7 (CH) |
| 2′ | 4.03 (m) | 75.2 (CH) | 3.16 (m) | 75.1 (CH) |
| 3′ | 4.21 (m) | 78.5 (CH) | 3.38 (m) | 77.9 (CH) |
| 4′ | 4.21 (m) | 71.8 (CH) | 3.38 (m) | 71.5 (CH) |
| 5′ | 4.01 (m) | 77.0 (CH) | 3.46 (m) | 76.8 (CH) |
| 6′ | 4.77 (dd, 11.2, 2.2) | 69.3 ($CH_2$) | 4.10 (dd, 11.6, 2.5) | 69.2 ($CH_2$) |
| | 4.32 (dd, 11.2, 5.5) | | 3.75 (dd, 11.6, 5.2) | |
| 1″ | 4.99 (d, 6.7) | 105.1 (CH) | 4.33 (d, 6.7) | 104.9 (CH) |
| 2″ | 4.47 (dd, 6.7, 8.3) | 72.3 (CH) | 3.61 (dd, 6.7, 8.5) | 72.4 (CH) |
| 3″ | 4.21 (m) | 74.2 (CH) | 3.54 (m) | 74.1 (CH) |
| 4″ | 4.33 (m) | 69.0 (CH) | 3.81 (m) | 69.3 (CH) |
| 5″ | 4.32 (dd, 11.2, 5.5) | 66.2 ($CH_2$) | 3.87 (dd, 12.5, 3.4) | 66.5 ($CH_2$) |
| | 3.79 (dd, 11.2, 1.5) | | 3.55 (br d, 12.5) | |

Note: Assignments in both solvents were based on the $^{1}H—^{1}H$ COSY, $^{13}C—^{1}H$ COSY, and HMBC spectra.

69.3 (C-6′) and 66.2 (C-5″), 11 oxygen-bearing methines ranging from δ 105.1 (C-1″) to 69.0 (C-4″), and 5 quaternary carbons. The carbon signals at δ 119.8 (C, C-6), 197.8 (C, C-7), 100.5 (CH, C-8), and 209.6 (C, C-9) were typical of an allenic group present in grasshopper ketone and its derivatives [7,8]. Acid hydrolysis of **1** afforded D-glucose and D-xylose, which were identified by HPLC analysis with an optical rotation detector in direct comparison of the retention time ($t_R$) and optical rotation (positive or negative) with those of authentic samples. Analysis of the $^{1}H—^{1}H$ COSY and $^{13}C—^{1}H$ COSY spectra revealed the connectivity from C-2 via C-3 to C-4 (—$CH_2$—CHO—$CH_2$—). In the HMBC spectrum, correlations from H-1″ to C-6′, $H_2$-6′ to C-1″, H-1′ to C-3, and H-3 to C-1′ clarified the inter-linkage of two sugar moieties, i.e. β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl (primverosyl) [9,10] and its attachment to C-3 (Figure 1). In addition, HMBC correlations from H-8 to C-6 and C-10, both $H_3$-11 and $H_3$-12 to C-1,




**1**

Figure 1. Structure of compound **1**.

C-2, and C-6, and $H_3$-13 to C-4, C-5, and C-6 led us to construct the planar structure of **1**. The relative configuration was determined by the NOESY spectrum, in which correlations between H-3 and $H_3$-11, and between $H_3$-12 and $H_3$-13 were observed. All measured in $CD_3OD$, the $\delta$ value of C-3 ($\delta$ 72.7) was identical to that of staphylionoside D [8] but different from that ($\delta$ 73.4) of glochidionionoside D [11], indicating **1** to be 3*S*,5*R* absolute configurations. Moreover, enzymatic hydrolysis of **1** with naringinase yielded grasshopper ketone (**1a**), which was identified by comparison of its spectral data with the reported values [7]. Thus, compound **1** was determined as grasshopper ketone 3-*O*-primveroside.

## 3. Experimental

### 3.1. General experimental procedures

Optical rotations were measured on a Horiba SEPA-300 digital polarimeter (Horiba, Kyoto, Japan); the UV spectrum was recorded on a Shimadzu UV-1600 spectrometer (Shimadzu, Kyoto, Japan); the IR spectrum was obtained on a Shimadzu FTIR-1800 spectrometer (Shimadzu); FAB-MS and HR-FAB-MS spectra were obtained on a JEOL JMS-SX 102A mass spectrometer (JEOL, Tokyo, Japan); EI-MS was recorded on a JEOL JMS-GCMATE mass spectrometer (JEOL); 1D and 2D NMR spectra were recorded on a JEOL JNM-LA 500 spectrometer (JEOL) using TMS as an internal standard. Analytic and preparative HPLC were carried out on a Shimadzu LC-6AD liquid chromatograph equipped with a Shimadzu RID-10A refractive index (RI) detector (Shimadzu) and YMC-Pack ODS-A columns (*S*-5 μm, 250 × 4.6 mm and 250 × 20 mm i.d., YMC, Kyoto, Japan). For column chromatography, silica gel BW-200 (150–300 mesh, Fuji Silysia Chemical, Aichi, Japan) and Chromatorex ODS DM1020T (100–200 mesh, Fuji Silysia Chemical) were used. Naringinase and Amberlite IRA-400 ($OH^-$ form) were purchased from Sigma-Aldrich, Tokyo, Japan. D-(+)-Glucose and D-(+)-xylose were obtained from Wako Pure Chemical Industries, Osaka, Japan.

### 3.2. Plant material

The whole herbs of *S. indica* cultivated in Guangxi, China, were purchased via Tochimoto Tenkaido Co., Osaka, Japan. The plant was botanically identified by Prof. Dian-Xiang Zhang in South China Botanical Garden, Chinese Academy of Sciences. A voucher specimen (WT041220) has been deposited at the Herbarium of South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China.

### 3.3. Extraction and isolation

The powder of air-dried whole herbs (9.9 kg) was extracted three times with methanol under reflux for 3 h each time. Evaporation of the solvent under reduced pressure gave a methanolic extract (764 g, 7.70%). The extract (725 g) was dissolved in water and sequentially fractionated with EtOAc and *n*-BuOH. The resultant *n*-BuOH-soluble fraction (95 g of the total 164 g) was subjected to silica gel (2.5 kg) column chromatography using a $CHCl_3$—MeOH gradient (10–50% MeOH, v/v) as eluents to afford eight fractions (Frs B1 − B8). Fr. B5 (12.5 g) was separated by Chromatorex ODS (350 g) column chromatography using a MeOH–$H_2O$ gradient (10–40% MeOH, v/v) as eluents to yield 10 fractions (Frs B5-1 − B5-10). Fr. B5-4 (0.51 g of the total 0.71 g) was purified by preparative HPLC with an RI detector using MeOH—$H_2O$ (25:75, v/v) as a mobile phase at the flow rate of 9 ml/min to furnish compound **1** ($t_R$ 38.2 min, 42.8 mg, 0.0011%).

#### 3.3.1. *Grasshopper ketone 3-O-primveroside (**1**)*

White amorphous powder; $[\alpha]_D^{20}$ − 83.5 (*c* 1.00, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$)

232 (4.09) nm; IR (KBr) $\nu_{max}$ 3450, 1945, 1673, 1392, 1250, 1168 $cm^{-1}$; $^1H$ NMR (500 MHz) and $^{13}C$ NMR (125 MHz) spectral data, see Table 1; FAB-MS *m/z* 541 $[M + Na]^+$ and 517 $[M - H]^-$; HR-FAB-MS *m/z* 541.2261 $[M + Na]^+$ (calcd for $C_{24}H_{38}O_{12}Na^+$, 541.2255).

### 3.3.2. *Acid hydrolysis of **1***

A solution of **1** (1.5 mg) in 1 M HCl (2.0 ml) was stirred at 80°C for 3 h. The cooled reaction mixture was neutralized with Amberlite IRA-400 ($OH^-$ form), and the filtrate was partitioned with EtOAc. The resultant aqueous layer was passed through a Sep-Pak Cartridge (Waters, MA, USA) and then subjected to HPLC analysis on a Shimadzu LC-6AD liquid chromatograph equipped with a Shodex OR-2 optical detector (Showa Denko, Tokyo, Japan) and a Kaseisorb LC $NH_2$-60-5 column (250 × 4.6 mm, i.d., Tokyo Kasei, Tokyo, Japan) using $CH_3CN–H_2O$ (85:15, v/v) as a mobile phase at the flow rate of 0.8 ml/min. Identification of the D-glucose and D-xylose from **1** was carried out by comparison of their retention times and optical rotations with those of authentic samples (D-glucose: $t_R$ 13.9 min, positive optical rotation; D-xylose: $t_R$ 17.4 min, positive optical rotation) [4,5].

### 3.3.3. *Enzymatic hydrolysis of **1***

A solution of **1** (8.7 mg) in 2.0 ml of 0.1 M acetate buffer (37% 0.1 M sodium acetate in 0.1 M acetic acid, v/v, pH 4.4) was treated with naringinase (6.5 mg) and stirred at 37°C for 24 h [12]. The reaction mixture was centrifuged at 4000 rpm for 10 min after 8 ml of EtOH was added. The supernatant was condensed *in vacuo* to give a residue, which was purified by preparative HPLC with a RI detector using $MeOH–H_2O$ (45:55, v/v) as a mobile phase at the flow rate of 9 ml/min to furnish **1a** ($t_R$ 21.4 min, 2.7 mg, 73% yield).

### 3.3.4. *Grasshopper ketone (**1a**)*

White amorphous powder; $[\alpha]_D^{20}$ −56.8 (*c* 0.15, MeOH); $^1H$ NMR ($C_5D_5N$, 500 MHz) $\delta$ 2.31 (1H, dd, $J$ = 12.5, 4.0 Hz, H-2α), 1.76 (1H, dd, $J$ = 12.5, 12.5 Hz, H-2β), 4.94 (1H, m, H-3), 2.79 (1H, dd, $J$ = 12.5, 3.5 Hz, H-4α), 1.83 (1H, dd, $J$ = 11.9, 12.5 Hz, H-4β), 5.92 (1H, s, H-8), 2.26 (3H, s, $H_3$-10), 1.63 (3H, s, $H_3$-11), 1.19 (3H, s, $H_3$-12), 1.59 (3H, s, $H_3$-13), 6.23 (br s, 3-OH), 6.75 (br s, 5-OH); $^{13}C$ NMR ($C_5D_5N$, 125 MHz) $\delta$ 36.4 (C, C-1), 50.5 ($CH_2$, C-2), 63.4 (CH, C-3), 50.9 ($CH_2$, C-4), 71.5 (C, C-5), 120.0 (C, C-6), 197.9 (C, C-7), 100.5 (CH, C-8), 209.8 (C, C-9), 26.4 ($CH_3$, C-10), 29.4 ($CH_3$, C-11), 32.1 ($CH_3$, C-12), 31.2 ($CH_3$, C-13) (assignments were based on the DEPT, $^1H—^1H$ COSY, HMQC, and HMBC spectra); EI-MS *m/z* (rel. int., %) 224 $[M]^+$(2), 209 (55), 191 (13), 181 (3), 163 (53), 149 (31), 145 (12), 123 (100), 109 (42), 105 (25), 77 (43).

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