A new 5H-purin-6-amine from the leaves of Sedum sarmentosum

Sunghun Cho¹ · Jaemin Lee¹ · Joyce P. Rodriguez¹ · Buom-Yong Ryu² · Chan Kyu Han³ · Sanghyun Lee¹

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Abstract Phytochemical constituents were isolated from Sedum sarmentosum leaves using open column chromatography and medium-pressure liquid chromatography. Their structures were identified as 2,4-pyrimidinedione (1), N-methylhydroxylamine (2), 5H-purin-6-amine (3), uridine (4), L-tyrosine (5), and L-prolyl-L-tyrosine (6) using mass spectrometry and ¹H- and ¹³C-nuclear magnetic resonance spectroscopic analysis. Among them, compound 3 (5H-purin-6-amine) was isolated for the first time from a natural source.

Keywords Chromatography · 5H-Purin-6-amine · Isolation · Sedum sarmentosum

Introduction

Sedum sarmentosum Bunge is a perennial herb distributed across Asia, Europe, and North America [1]. It is commonly known as Dolnamul in Korea. Fresh leaves of S. sarmentosum have been used in salads as an alternative to pepper because of their pungent taste. Moreover, traditionally, it has been used as a hepatoprotective medicinal plant in Asian countries [2]. Few studies have focused on the biological activities of S. sarmentosum [1]. S. sarmentosum shows unique angiotensin-converting enzyme inhibitory activity. Some flavonoids also show this activity [3]. Kim et al. [4] suggested that S. sarmentosum contains estrogens to promote a better life in menopausal women [4].

The objective of this study was to find new bioactive compounds from the leaves of S. sarmentosum. Therefore, our investigation was designed for the systematic isolation and identification of these valuable phytochemicals.

Materials and methods

Plant materials

Fresh S. sarmentosum leaves were cultivated at Juksanmyeon, Anseong, Republic of Korea.

Apparatus and chemicals

Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 500 NMR (Rheinstetten, Germany) spectrometer. Mass spectrometry (MS) was performed using a JEOL JMS-600 W (Tokyo, Japan) mass spectrometer. Optical rotations were measured on a Jasco P-2000 digital polarimeter (Tokyo, Japan). Medium-pressure liquid chromatographic separation was carried out on the Biotage (Uppsala, Sweden) system.

Extraction, fractionation, and isolation

The fresh S. sarmentosum leaves were dried for 3 days, and the powdered S. sarmentosum leaves (1.3 kg) were subjected to extraction with EtOH (8 L × 10) under reflux at...
65–75 °C. The filtrate was concentrated until dry in vacuo to afford a dark green EtOH extract (286.1 g). The EtOH extract (250.5 g) was suspended in H2O and then partitioned successively using n-hexane (57.8 g), CH2Cl2 (5.5 g), EtOAc (6.9 g), and n-BuOH (76.5 g). Among the fractions, a portion of the EtOAc fraction (5 g) was subjected to medium-pressure liquid chromatography (MPLC) with a gradient elution of CHCl3–MeOH (100:0→50:50) to yield 21 (EF1 to EF21) sub-fractions. EF9 sub-fraction was recrystallized under CHCl3–MeOH to yield compound 1. A portion of the n-BuOH fraction (60 g) was subjected to MPLC on silica gel using a gradient elution of CHCl3–MeOH (76.5 g). Among the fractions, a portion of the EtOAc fraction (5 g) was repeatedly subjected to MPLC on silica gel using a gradient elution of CHCl3–MeOH to obtain seven (BF6-1 to BF6-7) fractions, and BF6 fraction was repeatedly chromatographed on a Sephadex LH-20 column and eluted with CHCl3–MeOH (80:20→50:50) to obtain ten (BF1 to BF10) sub-fractions. BF9 sub-fraction was separated on a Sephadex LH-20 column and isolated compound 5. BF9-4 fraction was repeatedly chromatographed on a Sephadex LH-20 column to obtain seven (BF9-4-1 to BF9-4-7) and isolated compound 6. BF9 fraction was recrystallized under CHCl3–MeOH to yield compound 4. BF9-5 fraction was separated on a Sephadex LH-20 column and isolated compound 6.

**Table 1**  
| No. | δH | δC | HMBC |
|-----|----|----|------|
| 2   | 8.45 (s) | 146.1 | C-6 |
| 4   | 151.4 |
| 5   | 3.16 (s) | 48.6 |
| 6   | 149.3 |
| 8   | 8.46 (s) | 142.7 | C-4 |

**Results and discussion**

A chromatographic separation of the MeOH extract obtained six compounds (Fig. 1). The known compounds, 2,4-pyrimidinedione (1) [5], N-methylhydroxylamine (2) [6], uridine (4) [5], L-tyrosine (5) [7], and L-prolyl-L-tyrosine (6) [8], were identified by comparison with spectroscopic data from the studies.

Compound 3 was obtained as a white amorphous powder. A molecular ion peak was measured at m/z 135 [M]+ in the EI-MS, which corresponds to a molecular formula of C6H6N5 by HREI-MS (m/z 135.0546 [M]+, calcd. for 135.0545). 1H-NMR spectra of 3 showed a methine proton at δ 3.16 and the presence of two aromatic protons at δ 8.45 and 8.46. The 13C-NMR spectrum indicated 5 carbon resonances. The 13C-NMR spectrum showed a methane carbon at δ 48.5 and two olefin quaternary and two olefin methane carbons at δ 142.7, 146.1, 149.3 and 151.4. The structure of 3 was similar to that of adenine [9]. Thus, the structure of 3 was assigned as 5H-purin-6-amine via spectroscopic analysis.

Six compounds were isolated for the first time from S. sarmentosum leaves. According to previous studies,
compound 3 (5H-purin-6-amine) was isolated for the first time from a natural source.

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