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Novel megastigmanes with lipid accumulation inhibitory and lipid metabolism-promoting activities in HepG2 cells from *Sedum sarmentosum*☆

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ABSTRACT

Four novel megastigmanes, neosedumosides I (**1**), II (**2**), III (**3**), and IV (**4**) were isolated from the whole plant of *Sedum sarmentosum* (Crassulaceae). Absolute stereostructures of these constituents were determined on the basis of chemical and physicochemical evidence. Among them, **1**–**3** were found to show lipid accumulation inhibitory activity in HepG2 cells. Furthermore, **2** and **3** were found to also show lipid metabolism-promoting activity.

1. Introduction

During the course of our characterization studies on bioactive constituents from Chinese natural medicines,[1–5] we have reported the isolation and structural elucidation of 27 megastigmane constituents, including sarmentoic acid, sarmentol A, sedumosides A_1–A_6, B, C, D, E_1–E_3, F_1, F_2, and G–I, and four flavonol glycosides, sarmenosides I–IV from the whole plant of *Sedum sarmentosum* (Crassulaceae).[2–5] As a continuing study on this herbal medicine, we have isolated four novel bicyclic megastigmane glycosides, neosedumosides I (**1**), II (**2**), III (**3**), and IV (**4**). This paper deals with the isolation and structural elucidation of these new megastigmanes (**1**–**4**) and their lipid accumulation inhibitory and lipid metabolism-promoting activities.

2. Results and discussion

The MeOH-eluted fraction (72.0 g) from the whole plant of *S. sarmentosum*[3] was subjected to SiO_2 and ODS column chromatographies and finally HPLC (ODS column, eluted with CH_3CN–MeOH–H_2O solvent system) to furnish five novel megastigmane glycosides, neosedumosides I (**1**, 25.4 mg), II (**2**, 12.3 mg), III (**3**, 54.2 mg), and IV (**4**, 9.2 mg) (Chart 1).

Neosedumoside I (**1**), $[\alpha]_D^{25}$ +40.0 (MeOH), was obtained as an amorphous powder. Its IR spectrum showed absorption bands at 3389, 1653, and 1036 cm^{-1} ascribable to hydroxyl, α,β-unsaturated olefin, and ether functions, respectively. In the UV spectrum, an absorption maximum was observed at 241 nm (log ε 4.06 in MeOH) ascribable to the enone moiety. The EIMS of **1** showed a molecular ion peak at *m*/*z* 386 (M^+), and the molecular formula was determined as $C_{19}H_{30}O_8$ by high-resolution EIMS measurement. The ^1H and ^{13}C NMR spectra of **1** (CD_3OD, Tables 1 and 2) showed

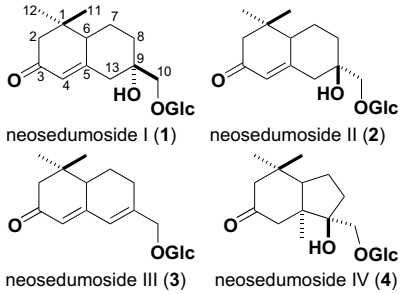

Chart 1. Structures of neosedumosides I–IV (**1**–**4**).

☆ See Ref. 1.

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Table 1
^{1}H NMR data for neosedumosides I (**1**), II (**2**), III (**3**), and IV (**4**) (at 500 MHz in CD_3OD, *J* values in Hz)

	1	2	3	4
2α	2.19 (d, 15.6)	2.19 (d, 15.6)	2.44 (d, 16.2)	1.88 (d, 17.4)
2β	2.25 (d, 15.6)	2.26 (d, 15.6)	2.17 (dd, 0.9, 16.2)	2.43 (d, 17.4)
3				
4(α)	5.83 (br s)	5.83 (br s)	5.79 (br s)	2.09 (d, 17.7)
4β				2.83 (d, 17.7)
6	2.23 (m)	2.19 (m)	2.50 (m)	1.80 (m)
7α	1.95 (m)	1.89 (m)	2.05 (m)	1.77 (2H, m)
7β	1.43 (m)	1.78 (m)	1.45 (m)	
8α	1.61 (ddd, 4.0, 13.1, 13.4)	1.74 (m)	2.40 (m)	1.64 (m)
8β	2.15 (m)	1.81 (m)	2.30 (m)	1.71 (m)
10	3.39, 3.78 (both d, 10.7)	3.40, 3.83 (both d, 10.1)	4.22, 4.43 (both d, 14.7)	3.48, 4.04 (both d, 10.5)
11	0.95 (s)	0.98 (s)	0.88 (s)	0.97 (s)
12	1.06 (s)	1.07 (s)	1.15 (s)	1.05 (s)
13	2.37 (d, 13.9) 2.70 (dd, 2.1, 13.9)	2.48 (d, 14.6) 2.54 (dd, 1.9, 14.6)	6.45 (s)	1.10 (s)
1′	4.25 (d, 7.6)	4.29 (d, 7.7)	4.29 (d, 7.7)	4.22 (d, 7.7)
2′	3.22 (dd, 7.7, 9.2)	3.23 (dd, 7.7, 9.2)	3.23 (m)	3.22 (dd, 7.7, 9.2)
3′	3.36 (m)	3.36 (m)	3.36 (dd, 8.5, 8.5)	3.35 (dd, 9.2, 9.2)
4′	3.26 (dd, 8.6, 8.6)	3.28 (m)	3.28 (m)	3.27 (m)
5′	3.25 (m)	3.28 (m)	3.26 (m)	3.26 (m)
6′	3.65 (dd, 5.5, 11.4) 3.83 (dd, 1.8, 11.4)	3.65 (dd, 5.5, 11.9) 3.87 (dd, 1.8, 11.9)	3.66 (dd, 5.5, 11.9) 3.86 (dd, 2.1, 11.9)	3.65 (dd, 5.8, 11.9) 3.86 (dd, 1.5, 11.9)

signals assignable to two methyls [δ 0.95, 1.06 (3H each, both s, H_3-11, 12)], four methylenes, a methine, a methylene bearing an oxygen function [δ 3.39, 3.78 (1H each, both d, *J*=10.7 Hz, H_2-10)], a quaternary carbon bearing an oxygen function (δ_C 74.5), a trisubstituted olefin [δ 5.83 (1H, br s, H-4), δ_C 125.5 (C-4), 165.7 (C-5)], and a conjugated carbonyl carbon (δ_C 202.5) together with those of a glucopyranosyl moiety [δ 4.25 (1H, d, *J*=7.6 Hz, H-1′)]. Acid hydrolysis of **1** with 1 M HCl liberated the D-glucose, which was identified by HPLC analysis using an optical rotation detector.[2–5] The bicyclic neomegastigmane skeleton of **1** was constructed on the basis of various NMR experiments.[6] Namely, the ^{1}H–^{1}H COSY experiments on **1** indicated the presence of two partials written in bold lines, while in the HMBC experiments, long range correlations were observed between the following proton and carbon pairs: H_2-2 and C-1, 3, 4; H_2-4 and C-2, 5, 6, 13; H-6 and C-1, 4, 5; H_2-7 and C-5; H_2-8 and C-6; H_2-10 and C-8, 9, 13; H_3-11 and C-1, 2, 6, 12; H_3-12 and C-1, 2, 6, 11; H-1′ and C-10 (Fig. 1). Next, the relative stereostructure of **1** was clarified by the NOESY experiment, in which

Table 2
^{13}C NMR data for **1**–**4** (at 125 MHz)

	1[a]	2[a]	3[a]	4[a]	4[b]
1	35.8	35.7	37.3	34.7	34.2
2	51.6	51.3	54.7	48.8	48.7
3	202.5	202.5	202.8	217.3	212.1
4	125.5	125.6	123.8	46.8	46.1
5	165.7	167.1	159.7	48.8	47.9
6	49.3	50.0	46.9	56.9	55.6
7	24.5	23.7	23.4	26.7	25.8
8	35.2	34.1	28.2	34.7	34.2
9	74.5	74.6	151.8	84.8	83.7
10	74.5	79.0	72.7	75.3	75.2
11	24.3	24.6	20.3	29.3	28.0
12	28.9	28.9	29.0	30.4	30.0
13	46.1	46.1	126.5	29.5	28.7
1′	104.9	105.0	104.0	105.0	106.0
2′	75.2	75.2	75.1	75.1	75.2
3′	78.0	77.9	78.1	78.0	78.8
4′	71.6	71.6	71.6	71.7	71.6
5′	77.9	78.0	78.0	78.1	78.7
6′	62.7	62.7	62.8	62.8	62.7

[a] In CD_3OD.
[b] In pyridine-d_5.

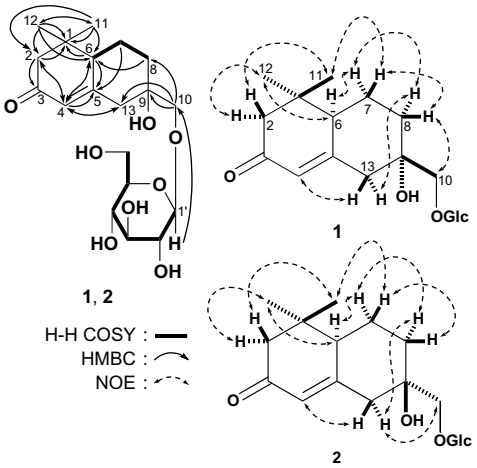

Figure 1. ^{1}H–^{1}H COSY, HMBC, and NOE correlations of **1** and **2**.

correlations were observed between the following proton pairs: H-2α and H_3-12; H-2β and H_3-11; H-4 and H-13β; H-6 and H-7α, H_3-12; H-7α and H-8α; H-7β and H-8β, H_3-11; H-8α and H-13α; H-8β and H_2-10 (Fig. 1). On the basis of above-mentioned evidence, the relative stereostructure of **1** was elucidated as shown in Figure 1.

Neosedumoside II (**2**) was isolated as an amorphous powder with positive optical rotation ($[\alpha]_D^{25}$ +39.6 (MeOH)). The EIMS of **2** showed a molecular ion peak at *m*/*z* 386 (M^+), and the molecular formula, $C_{19}H_{30}O_8$, was found to be the same as that of **1** by high-resolution EIMS measurement. The ^{1}H and ^{13}C NMR spectroscopic properties of **2** (CD_3OD, Tables 1 and 2) were quite similar to those of **1**. That is, **2** showed signals due to two methyls [δ 0.98, 1.07 (3H each, both s, H_3-11, 12)], four methylenes, a methine, a methylene bearing an oxygen function [δ 3.40, 3.83 (1H each, both d, *J*=10.1 Hz, H_2-10)], a quaternary carbon bearing an oxygen function (δ_C 79.0), a trisubstituted olefin [δ 5.83 (1H, br s, H-4), δ_C 125.6 (C-4), 167.1 (C-5)], and a conjugated carbonyl carbon (δ_C 202.5) together with those of a glucopyranosyl moiety. On acid hydrolysis with 1 M HCl, **2** liberated the D-glucose. The planar structure of **2** was characterized to be the same as that of **1** by means of ^{1}H–^{1}H COSY and HMBC experiments as shown in Figure 1. The NOESY spectrum of **2** showed distinct correlation between H-13α and H_2-10 (Fig. 1). Thus, **2** was clarified to be the stereoisomer of **1** at the 10-position.

Neosedumoside III (**3**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{27}$ −63.7 (MeOH)). The positive-ion FABMS showed a quasimolecular ion peak at *m*/*z* 391 $(M+Na)^+$ and its molecular formula, $C_{19}H_{28}O_7$, was determined by high-resolution FABMS measurement. In the UV spectrum, an absorption maximum was observed at 290 nm (log ε 4.12 in MeOH), which was suggestive of a hetero-annular diene chromophore. The IR spectrum showed absorption bands due to hydroxyl, olefin, and ether groups at 3649, 1653, and 1076 cm^{-1}, respectively. ^{1}H and ^{13}C NMR spectra of **3** (CD_3OD, Tables 1 and 2) showed signals assignable to two methyls [δ 0.88, 1.15 (3H each, both s, H_3-11, 12)], three methylenes, a methine, a methylene bearing an oxygen function [δ 4.22, 4.43 (1H each, both d, *J*=14.7 Hz, H_2-10)], two trisubstituted olefins [δ 5.79, 6.45 (1H each, both br s, H-4, 13), δ_C 123.8 (C-4), 126.5 (C-13), 151.8 (C-9), 159.7 (C-5)], and a conjugated carbonyl carbon (δ_C 202.8) together with those of a glucopyranosyl moiety. The acid hydrolysis of **3** liberated the D-glucose. Connectivities of the quaternary carbons and the β-D-glucopyranosyl part were elucidated on the basis of various NMR measurements, and the relative stereostructure of **3** was unambiguously clarified as shown in Figure 2 by the NOESY experiment.