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Three new triterpene saponins from *Actinostemma lobatum* MAXIM and their cytotoxicity *in vitro*

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ABSTRACT

Three new triterpene saponins, lobatoside O (**1**), actinostemmoside I (**2**) and actinostemmoside J (**3**), were isolated from the herb of *Actinostemma lobatum* MAXIM. Their structures were elucidated by means of extensive chemical and spectroscopic methods. In addition, cytotoxic activities toward HCT-116, HT-29, MCF-7 and A549 cell lines were tested by the MTT method.

1. Introduction

Actinostemma lobatum MAXIM, a wildlife plant of Cucurbitaceae family, is widely distributed in Southeast Asian countries such as Japan, India, Korea and China. For centuries, it has been used for treatment of arthritis, cardiovascular and diuretic diseases as a folk remedy (Kim et al., 1989). In recent years, *A. lobatum* has received considerable attention because of its potent anticancer activity, and the saponins have been reported as the main bioactive components in it (Li et al., 2012). To learn the relationship between the structures and the anticancer activities of the triterpene saponins of this plant is important. Dammarane-type and oleanane-type glycosides are the major classes of saponins isolated from *A. lobatum*. In the previous study, we had isolated and identified six cyclic bisdesmoside saponins from *A. lobatum* (Li et al., 2012). In the present study, we investigated saponins from this plant except the cyclic-bisdesmoside-type saponins, seven compounds (Fig. 1) were isolated including three new triterpene saponins **1**–**3** and the structures were elucidated. Furthermore, the cytotoxic activities toward HCT-116, HT-29, MCF-7 and A549 cell lines *in vitro* were evaluated.

2. Results and discussion

Compound **1**, white amorphous powders, showed a peak at *m/z* 977.5085 $[M+Na]^+$ in the HR-TOF-ESI-MS, indicating the molecular formula $C_{50}H_{84}O_{16}$. The IR spectrum (KBr) showed peak at 3439 cm^{-1} (OH), 1702 cm^{-1} (C=O). The ^1H NMR spectrum (Table 1) displayed eight methyl groups 0.82 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 0.93 (3H, s), 1.09 (3H, s), 1.25 (3H, s), 1.28 (3H, s), 1.71 (3H, d, J = 6.0 Hz), a methoxy groups δ 3.80 (3H, s), three anomaric protons δ 4.91 (1H, d, J = 7.8 Hz), 6.32 (1H, br. s), 6.34 (1H, d, J = 8.4 Hz) and a proton on the trisubstituted olefin part δ 5.42 (br s). The carbon signals assignable to the aglycone part in the ^{13}C NMR spectrum (Table 1) were almost the same as those of lobatoside K (Fujioka et al., 1992), an oleanolic acid 3, 28-O-bisdesmoside, except that the sugar moieties of **1** were different from those of lobatoside K. According to the splitting pattern of the anomeric proton signals in ^1H NMR spectrum and the results of 1D and 2D NMR, the part of α-rhamnopyranosyl-(1 → 2)-β-glucopyranosyl should be linked at C3-O, and C28-O connected with β-glucuronic acid methyl ester. Following key long-range correlations in HMBC could testify its structure: between δ 4.91 (1H, d, J = 7.8 Hz, Glc H-1) and δ 89.3 (C-3), between δ 6.32 (1H, br. s, Rha H-1′) and δ 82.0 (Glc C-2), between δ 6.34 (1H, d, J = 8.4 Hz, GluA H-1″) and δ 176.4 (C-28), between δ 3.80 (3H, s, OCH_3) and δ 170.7 (C-GluA C-6″). The stereochemistry of sugars was determined to be L-rhamnose, D-glucuronic acid and D-glucose by acid hydrolysis and further by GC analysis of the trimethylsilyl ethers derivatives (the monosaccharides of the acid hydrolysate t_R min: 9.56, 10.81 and 22.79, and the standard monosaccharides were subjected

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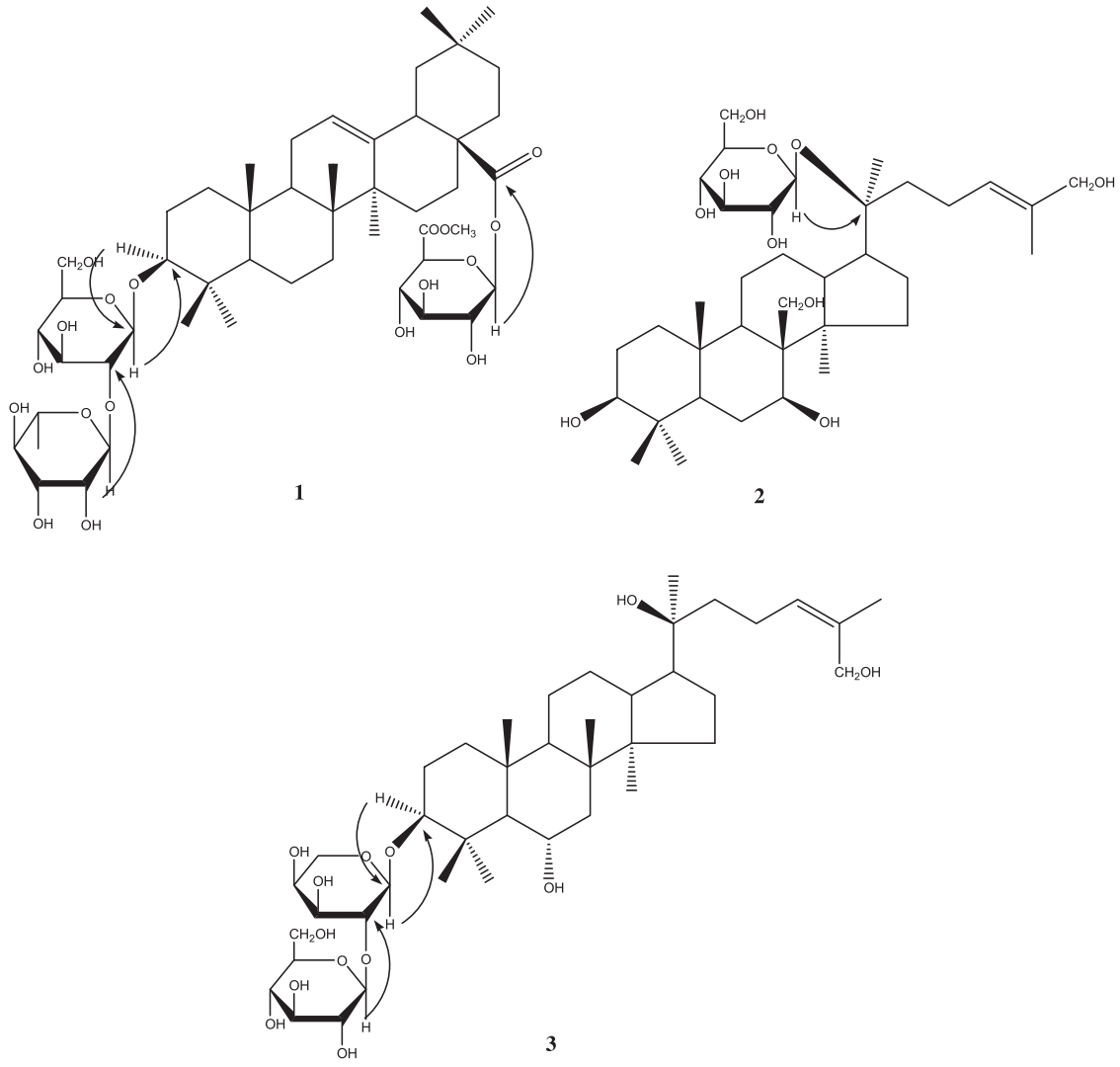

Fig. 1. Structures and key HMBC correlations of **1**–**3**.

to the same reaction and GC analysis under the same condition). Combining the analysis of the above data, the structure of compound **1** was elucidated as 3β-hydroxy-oleanolic acid 3-O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl- 28-O-β-D-glucuronic acid methyl ester (Fig. 1), named lobatoside O.

Compound **2** was obtained as white amorphous powders from aqueous methanol. Its molecular formula, $C_{36}H_{62}O_{10}$, was determined by the $[M + Na]^+$ quasimolecular ion peak at *m/z* 677.4237 (Calcd. 677.4241) in the HR-TOF-ESI-MS. The IR spectrum showed the absorption bands of hydroxyl group (3421 cm^{-1}) and carbonyl group (1695 cm^{-1}). The ^1H NMR spectrum (Table 2) revealed the presence of six methyl groups at δ 1.06 (3H, s), 1.09 (3H, s), 1.22 (3H, s), 1.22 (3H, s), 1.57 (3H, s), 1.88 (3H, s), two oxygen-bearing methane at δ 3.47 (1H, m), 3.89 (1H, m), two pair of oxygen-bearing methylene, one at δ 4.29 (2H, m), the other at δ 4.73 (d, J = 11.4 Hz), 4.56 (d, J = 11.4 Hz), an anomaric proton at δ 5.11 (1H, d, J = 7.8 Hz), an olefinic proton at δ 5.79 (1H, t, J = 7.2 Hz). The ^{13}C NMR spectrum (Table 1) showed 36 carbon signals, including a set of β-glucopyranosyl signals and 30 signals due to a dammarane-type triterpene aglycone, which were superimposable on those of actinostemmoside C (Iwamoto et al., 1987) except for the side-chain part. Careful comparison of the NMR spectral data of the aglycone of compound **2** with those of actinostemmoside C and Kizuta saponin K9 (Kizu et al., 1985) indicated that compound **2** possessed the same tetracyclic moiety as actinostemmoside C and the same side-chain moiety as Kizuta saponin K9. Namely, the aglycone of compound **2** was established to be (20S)-3β, 7β, 18, 20, 26-pentahydroxy-dammar-24-ene. The β-glucopyranosyl bonding to C20-O of the aglycone was characterized by HMBC (Fig. 1), in which the long-range correlation was detected: from the anomaric protons at δ 5.11 (1H, d, J = 7.8 Hz) to C-20 at δ 82.2. The sugar was determined to be D-glucose by GC analysis as described above. Accordingly, compound **2** was elucidated as (20S)-3β, 7β, 18, 20, 26-pentahydroxy-dammar-24-ene 20-O-β-D-glucopyranoside, named actinostemmoside I.

Compound **3** was obtained as colorless needles. Its molecular formula was deduced to be $C_{41}H_{70}O_{13}$ by positive mass spectrometry (HR-TOF-ESI-MS) data at *m/z* 793.4702 $[M + Na]^+$ (Calcd. for $C_{41}H_{70}NaO_{13}$, 793.4714). In the ^1H NMR spectrum (Table 1) of **3**, the following signals could be observed: seven tertiary methyls at δ 0.91 (3H, s), 0.98 (3H, s), 1.05 (3H, s), 1.38 (3H, s), 1.45 (3H, s), 1.92 (3H, s), 2.03 (3H, s), two hydroxymethylene at δ 3.30 (1H, dd, J = 12.0, 4.2 Hz), 4.30 (1H, m), two anomaric protons at δ 5.09 (1H, d, J = 5.4 Hz), 5.18 (1H, d, J = 7.2 Hz), and an olefinic proton at δ 5.49 (1H, t, J = 7.2 Hz). The ^{13}C NMR spectrum (Table 1) showed a group of (20S)-3β, 6α, 20, 27-tetrahydroxy-dammar-24-ene signals at δ 136.1 (C-25), 127.8 (C-24), 89.5 (C-3), 74.1 (C-20), 67.5 (C-6), 61.8 (C-5), 60.9 (C-27), 50.7 (C-17), 21.9 (C-26), indicating its aglycone