# Triterpenoid Saponins from *Gypsophila altissima* L.

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Received October 27, 2009; accepted November 30, 2009; published online December 4, 2009

**Two new triterpenoid saponins (1, 2) were isolated from the roots of *Gypsophila altissima* L. (Caryophyllaceae), together with a known compound (3). The structures of new saponins were established as quillaic acid 3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucuronopyranoside (1), and 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-(6-*O*-acetyl)-*β*-D-glucopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→4)]-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside (2), on the basis of various spectroscopic analyses (including heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC), nuclear Overhauser effect spectroscopy (NOESY), total correlation spectroscopy (TOCSY), and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS)) and chemical degradations.**

**Key words** *Gypsophila altissima* L.; Caryophyllaceae; triterpenoid saponin

The *Gypsophila* genus belonging to the Caryophyllaceae family includes about 150 species around the world, which are widely distributed in Asia and Europe. Of these, more than 18 species grow in mainland China, especially in Xinjiang province,[1] many of which have applications in either traditional Chinese medicine (TCM) or folklore herbs to treat fever, consumptive disease, and infantile malnutrition syndrome.[2] Triterpenoid saponins with biological activities have been reported in our previous phytochemical investigations from this genus.[3,4] *G. altissima*, a perennial herbaceous plant, about 50—80 cm tall, grows at the mountain slopes, valley grasslands, floodlands and ditches at 1350—2450 m above the sea level. Up to now, there is no report of the chemical study about this plant. As a part of our search for bioactive saponins from Chinese herbs, this paper presents the isolation and structure elucidation of two new saponins (**1**, **2**) from *G. altissima*.

## Results and Discussion

Compound **1** was obtained as a white amorphous powder. $[\alpha]_D^{25}$ +3.5 ($c$=0.13, MeOH). The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) showed a pseudo molecular ion peak at $m/z$ 793.4038 $[M-H]^-$ (Calcd for $C_{41}H_{61}O_{15}$: 793.4015) corresponding to the molecular formula of $C_{41}H_{62}O_{15}$. Its IR spectrum revealed absorption bands at 3419 $cm^{-1}$ (OH), 1724 $cm^{-1}$ (C=O), 1678 $cm^{-1}$ (C=C), and 1042 $cm^{-1}$ (C–O–C). On acid hydrolysis with 2 M HCl, **1** afforded sugar moieties that identified as D-glucuronic acid and D-xylose in the ratio of 1 : 1 based on the GC-MS analysis of their chiral derivatives,[5] and an aglycone which was identified as quillaic acid from the $^1$H- and $^{13}$C-NMR (Table 1) referring to the reported data.[6] The $^{13}$C-NMR chemical shifts of C-3 ($\delta$ 81.9) and C-28 ($\delta$ 179.8) indicated that **1** was a monodesmosidic glycoside in which the sugar moiety was connected at C-3.[7] The $^1$H- and $^{13}$C-NMR data (Table 2) of the monosaccaride residues were assigned starting from the readily identifiable anomeric protons by means of the total correlation spectroscopy (TOCSY), nuclear Overhauser effect spectroscopy (NOESY), heteronuclear single quantum correlation (HSQC), and heteronuclear multiple bond correlation (HMBC) spectra obtained for this compound. The two sugar anomeric carbons were detected at

Table 1. $^{13}$C-NMR Data ($\delta$) for the Aglycone Moieties of Compounds **1** and **2** (150 MHz, $C_5D_5N$)

| Carbon | 1 | 2 | Carbon | 1 | 2 |
|---|---|---|---|---|---|
| 1 | 37.9 | 37.9 | 16 | 74.1 | 73.8 |
| 2 | 25.1 | 25.2 | 17 | 48.6 | 48.9 |
| 3 | 81.9 | 84.1 | 18 | 41.2 | 41.7 |
| 4 | 55.4 | 54.9 | 19 | 47.5 | 48.7 |
| 5 | 46.9 | 46.7 | 20 | 30.9 | 30.4 |
| 6 | 20.3 | 20.4 | 21 | 35.8 | 36.0 |
| 7 | 32.6 | 31.2 | 22 | 32.8 | 32.8 |
| 8 | 39.9 | 40.0 | 23 | 206.4 | 210.0 |
| 9 | 47.1 | 47.5 | 24 | 10.2 | 10.9 |
| 10 | 37.9 | 36.0 | 25 | 15.4 | 15.7 |
| 11 | 23.5 | 23.5 | 26 | 17.2 | 17.1 |
| 12 | 121.9 | 121.5 | 27 | 27.1 | 27.0 |
| 13 | 145.0 | 144.5 | 28 | 179.8 | 175.8 |
| 14 | 42.0 | 42.0 | 29 | 33.2 | 33.1 |
| 15 | 35.9 | 36.2 | 30 | 24.5 | 24.1 |

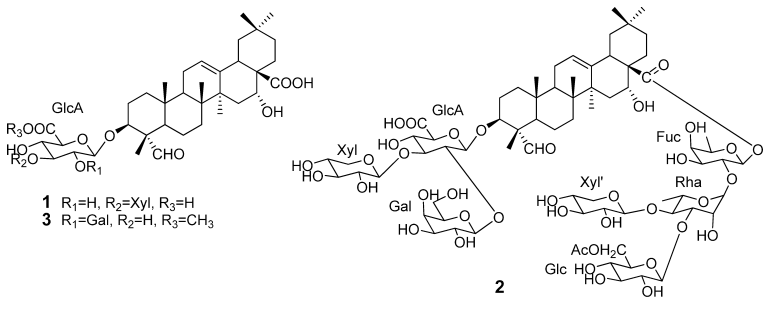


Fig. 1. The Structures of Compounds **1**—**3**

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Table 2. $^{13}$C- and $^{1}$H-NMR Data for Sugar Moieties of Compounds **1** and **2** ($C_5D_5N$)[a,b]

| **1** | | | **2** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$, Hz) | | $\delta_C$ | $\delta_H$ ($J$, Hz) | | $\delta_C$ | $\delta_H$ ($J$, Hz) |
| 3-*O*-GlcA | | | 3-*O*-GlcA | | | 28-*O*-Fuc | | |
| 1 | 104.7 | 4.93 (7.8) | 1 | 103.7 | 4.89 (7.8) | 1 | 94.8 | 5.90 (8.4) |
| 2 | 74.5 | 4.51 | 2 | <u>78.4</u> | 4.35 | 2 | <u>74.8</u> | 4.65 |
| 3 | <u>85.9</u> | 4.29 | 3 | <u>85.6</u> | 4.22 | 3 | 75.1 | 4.17 |
| 4 | 71.1 | 4.51 | 4 | 71.1 | 4.42 | 4 | 73.0 | 3.96 |
| 5 | 74.2 | 4.03 | 5 | 77.1 | 4.49 | 5 | 72.4 | 3.99 |
| 6 | 172.0 | | 6 | 171.8 | | 6 | 16.7 | 1.48 (6.6) |
| Xyl | | | Gal | | | Rha | | |
| 1 | 106.0 | 5.33 (7.2) | 1 | 104.0 | 5.50 (8.4) | 1 | 100.6 | 6.41 (s) |
| 2 | 75.3 | 3.97 | 2 | 73.5 | 4.45 | 2 | 71.3 | 5.01 |
| 3 | 78.0 | 4.15 | 3 | 75.2 | 4.14 | 3 | 83.0 | 4.65 |
| 4 | 70.8 | 4.13 | 4 | 69.9 | 4.54 | 4 | 78.4 | 4.51 |
| 5 | 67.2 | 3.68, 4.31 | 5 | 76.4 | 3.89 | 5 | 68.0 | 4.54 |
| | | | 6 | 61.4 | 4.42, 4.49 | 6 | 18.4 | 1.60 |
| | | | Xyl | | | Xyl′ | | |
| | | | 1 | 104.4 | 5.31 (6.6) | 1 | 105.4 | 5.40 (7.8) |
| | | | 2 | 75.6 | 3.88 | 2 | 76.3 | 3.95 |
| | | | 3 | 78.4 | 4.11 | 3 | 78.9 | 4.01 |
| | | | 4 | 70.6 | 4.10 | 4 | 71.5 | 4.09 |
| | | | 5 | 67.1 | 3.64, 4.21 | 5 | 66.9 | 3.32, 4.11 |
| | | | | | | Glc | | |
| | | | | | | 1 | 104.7 | 5.25 (7.8) |
| | | | | | | 2 | 75.0 | 3.90 |
| | | | | | | 3 | 78.0 | 4.07 |
| | | | | | | 4 | 71.1 | 3.89 |
| | | | | | | 5 | 77.8 | 3.95 |
| | | | | | | 6 | 64.1 | 4.88, 4.63 |
| | | | | | | <u>C</u>OCH$_3$ | 171.8 | |
| | | | | | | CO<u>CH$_3$</u> | 20.8 | 2.14 (s) |

*a*) The assignments were based upon $^{1}$H-NMR, $^{13}$C-NMR, HSQC, HMBC and NOESY spectra. *b*) $^{13}$C chemical shifts of substituted residues are underlined.

$\delta_C$ 104.7 and 106.0 in $^{13}$C-NMR spectrum, attached to protons at $\delta_H$ 4.93 (d, $J$=7.8 Hz) and 5.33 (d, $J$=7.2 Hz), respectively, in the HSQC experiment. The $\beta$-anomeric configurations of the D-glucuronic acid and D-xylose units were determined from their $^{3}J_{H1,H2}$ coupling constants (7—8 Hz).[8] The sequence of the sugar residue was subsequently determined by HMBC experiment. The long-range correlations between C-3 ($\delta_C$ 81.9) of the aglycone and H-1 ($\delta_H$ 4.93) of GlcA-1, C-3 ($\delta_C$ 85.9) of GlcA and H-1 ($\delta_H$ 5.33) of Xyl, indicated that the disaccharide moiety linked to the quillaic acid at C-3 was determined to be $\beta$-D-xylopyranosyl-(1→3)-$\beta$-D-glucuronopyranosyl. On the basis of the above results, the structure of **1** was established as quillaic acid 3-*O*-$\beta$-D-xylopyranosyl-(1→3)-$\beta$-D-glucuronopyranoside.

Compound **2** was obtained as an amorphous powder. $[\alpha]_D^{25}$ −13.0 ($c$=0.02, MeOH). It revealed an $[M-H]^-$ ion peak at $m/z$ 1583.6809 (Calcd for $C_{72}H_{111}O_{38}$: 1583.6758) in the negative HR-ESI-MS in agreement with the molecular formula $C_{72}H_{112}O_{38}$. The IR spectrum showed absorptions at 3443 cm$^{-1}$ (OH) and 1680 cm$^{-1}$ (C=C). Acid hydrolysis of **2** afforded quillaic acid as the aglycone, and D-glucuronic acid, D-galactose, D-xylose, D-fucose, L-rhamnose and D-glucose in the ratio of 1 : 1 : 2 : 1 : 1 : 1 based on the GC-MS analysis of their chiral derivatives. The $\beta$-anomeric configurations for the D-glucuronopyranosyl, D-galactopyranosyl, D-xylopyranosyl, D-glucopyranose and D-fucopyranosyl moieties were determined from their large $^{3}J_{H1,H2}$ coupling constants (6.6—8.0 Hz). And the $\alpha$-anomeric configuration of L-rhamnose was judged by its C-5 ($\delta_C$ 68.0).[9] The downfield $^{13}$C-NMR chemical shift at $\delta_C$ 84.1 and the upfield $^{13}$C-NMR chemical shift at $\delta_C$ 175.8 suggested that **2** was a bidesmosidic saponin with glycosidic linkages at C-3 through an ether bond and at C-28 through an ester bond. The anomeric proton signals were at $\delta_H$ 6.41 (s), 5.90 (d, $J$=8.4 Hz), 5.50 (d, $J$=8.4 Hz), 5.40 (d, $J$=7.8 Hz), 5.31 (d, $J$=6.6 Hz), 5.25 (d, $J$=7.8 Hz), and 4.89 (d, $J$=7.8 Hz), with the corresponding anomeric carbon signals at $\delta$ 100.6, 94.8, 104.0, 105.4, 104.4, 104.7 and 103.7, according to HSQC spectrum respectively. The linkage of the sugar units at C-3 of the aglycone was established from the following HMBC correlations: H-1 ($\delta_H$ 5.31) of xylose with C-3 ($\delta_C$ 85.6) of glucuronic acid, H-1 ($\delta_H$ 5.50) of galactose with C-2 ($\delta_C$ 78.4) of glucuronic acid, and H-1 of glucuronic acid ($\delta_H$ 4.89) with C-3 ($\delta_C$ 84.1) of the aglycone. The sugar chain at C-28 was established from the HMBC correlations observed: H-1 ($\delta_H$ 5.40) of xylose′ with C-4 ($\delta_C$ 78.4) of rhamnose, H-1 ($\delta_H$ 5.25) of glucose with C-3 ($\delta_C$ 83.0) of rhamnose, H-1 ($\delta_H$ 6.41) of rhamnose with C-2 ($\delta_C$ 74.8) of fucose, and H-1 ($\delta_H$ 5.90) of fucose with C-28 ($\delta_C$ 175.8) of the aglycone. Since the chemical shift of C-2 of GlcA is same as that of C-4 of Rha, their linkages of sugars could be finally determined from the NOESY experiment. The cross-peak between the H-2 ($\delta_H$ 4.35) of GlcA and H-1 ($\delta_H$ 5.50) of Gal indicated that the Gal moiety was linked to C-2 of GlcA. Similarly, the Xyl′ was linked to C-4 of the Rha deduced from correlation between the H-4 ($\delta_H$ 4.51) of Rha and H-1 ($\delta_H$ 5.40) of Xyl′ (Fig. 2). The structural fragement deduced above had been reported,[6] whose molecular weight was 1542, referring to the quasi-molecular ion mass

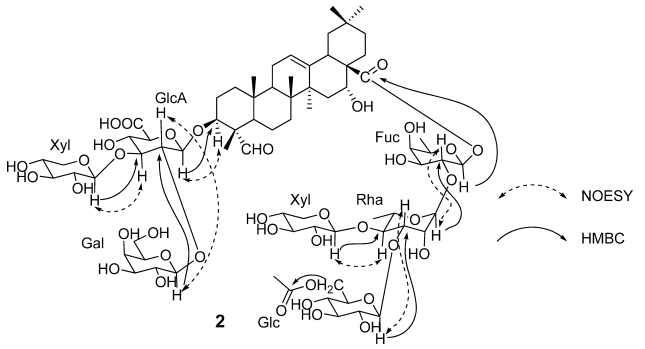


Fig. 2. Selected NOESY and HMBC Correlations for Compound **2**

of **2**, an extra 42 mass unit revealed the presence of an acetyl group combining with the information of the $\delta_C$ 20.8, 171.8 and $\delta_H$ 2.14. The downfield signals of Glc-6 at $\delta_H$ 4.88 (br d, $J$=12.0 Hz), 4.63 (br d, $J$=12.0 Hz) indicated that the location of the acetyl group was at this position, which was further confirmed by the correlations between these two protons and the carbonyl carbon ($\delta_C$ 171.8) of acetyl group in the HMBC spectrum, respectively. From the above evidences, the structure of **2** was elucidated as 3-*O*-$\beta$-D-galactopyranosyl-(1→2)-[$\beta$-D-xylopyranosyl-(1→3)]-$\beta$-D-glucuronopyranosyl quillaic acid 28-*O*-(6-*O*-acetyl)-$\beta$-D-glucopyranosyl-(1→3)-[$\beta$-D-xylopyranosyl-(1→4)]-$\alpha$-L-rhamnopyranosyl-(1→2)-$\beta$-D-fucopyranoside.

Compound **3** was determined as 3-*O*-$\beta$-D-galactopyranosyl-(1→2)-6-*O*-methyl-$\beta$-D-glucuronopyranosyl quillaic acid first obtained as the hydrolyzate of lucyoside N.[10]

## Experimental

**General Experimental Procedures** Optical rotations were measured with a JASCO P-1020 polarimeter (cell length: 1.0 dm). IR (KBr-disks) spectra were recorded by Brucker Tensor 27 spectrometer. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESI-MS) and a G1969A TOF MS (HR-ESI-MS), respectively. 1D and 2D NMR spectra were recorded in $C_5D_5N$ at 300 K on Bruker ACF-600 NMR ($^1$H: 600 MHz, $^{13}$C: 150 MHz) spectrometers, in which coupling constants were given in Hz. Gas chromatography was done on Varian CP-3800 Gas Chromatograph equipped with a Saturn 2200 Mass detector (detection temperature 220 °C). Column: CP-sil 5 CB capillary column (30 m, 0.25 mm i.d., 0.25 $\mu$m). Column temperature: 150—260 °C with the rate of 8 °C/min, and the carrier gas was He (0.8 ml/min), split ratio 1/10, injection temperature: 250 °C. Injection volume: 0.5 $\mu$l. All solvents used were of analytical or chromatographic grade (Jiangsu Hanbang Sci. & Tech. Co., Ltd). TLC was performed on precoated silica gel 60 $F_{254}$ plates (Qingdao Haiyang Chemical Co., Ltd.), and detection was achieved by 10% $H_2SO_4$–EtOH for saponins. Sephadex LH-20 (20×100 mm, Pharmacia), macroporous resin D101 (pore size B 13—14 nm, 26—60 mesh), and ODS-$C_{18}$ (40—63 $\mu$m, Fuji) were used for column chromatography. Preparative HPLC was carried out using Agilent 1100 Series with Shim-park RP-$C_{18}$ column (200×20 mm i.d.) and 1100 Series Multiple Wavelength detector.

**Plant Material** *G. altissima* was collected from Zhaosu County, Xinjiang Province, People's Republic of China, in August 2007, and the botanical origin of material was identified by Prof. Rena Kasimu, College of Pharmaceutical Sciences, Xinjiang Medical University, China. The voucher specimens (No. 070806) were deposited at the Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, China.

**Extraction and Isolation** The air-dried plants (5 kg) were extracted with 70% aqueous ethanol (v/v) three times (10 l, 2 h each) under reflux. After evaporation, the residue was suspended in $H_2O$ and partitioned between EtOAc and $H_2O$. Then the water layer was chromatographed over a macroporous resin D101 column eluted with 30%, 70% and 100% EtOH, respectively. The 70% EtOH portion was fractionated by MCI (MeOH/$H_2O$, 3 : 7, 5 : 5, 6 : 4, 7 : 3 and 10 : 0) to give five fractions (Fractions 1—5). Fraction 4 was further subjected to repeated RP-$C_{18}$ column with MeOH–$H_2O$ (4 : 6→9 : 1) and then the eluents of fraction 4.2 (MeOH–$H_2O$, 7 : 3) were further separated by HPLC (MeCN–0.05% TFA in $H_2O$, 35 : 65, UV detection at 210 nm), to yield **1** (20 mg) and **3** (25 mg) respectively. Fraction 2 was subjected to RP-$C_{18}$ column with MeOH–$H_2O$ (4 : 6→8 : 2) to give 3 fractions and compound **2** (9 mg) was obtained by further HPLC purification (MeCN–0.05% TFA in $H_2O$, 30 : 60, UV detection at 210 nm) from fraction 2.2.

Compound **1**: White amorphous powder; $[\alpha]_D^{25}$ +3.5 ($c$=0.13, MeOH); IR (KBr) cm$^{-1}$: 3419, 2948, 1724, 1678, 1447, 1387, 1080, 1042; ESI-MS $m/z$: 793 $[M-H]^-$; HR-ESI-MS $m/z$ 793.4038 $[M-H]^-$ (Calcd for $C_{41}H_{61}O_{15}$: 793.4015); $^1$H-NMR ($C_5D_5N$, 600 MHz) and $^{13}$C-NMR ($C_5D_5N$, 150 MHz) are given in Tables 1 and 2.

Compound **2**: White amorphous powder; $[\alpha]_D^{25}$ −13.0 ($c$=0.02, MeOH); IR (KBr) cm$^{-+}$: 3443, 2925, 1680, 1642, 1139; ESI-MS $m/z$: 1584 $[M-H]^-$; HR-ESI-MS $m/z$ 1583.6809 $[M-H]^-$ (Calcd for $C_{72}H_{111}O_{38}$: 1583.6758); $^1$H-NMR ($C_5D_5N$, 600 MHz) and $^{13}$C-NMR ($C_5D_5N$, 150 MHz) are given in Tables 1 and 2.

Compound **3**: White amorphous powder; $[\alpha]_D^{25}$ 0 ($c$=0.15, MeOH); IR (KBr) cm$^{-1}$: 3424, 2948, 1724, 1681, 1142, 1080; ESI-MS $m/z$: 837 $[M-H]^-$; $^1$H-NMR ($C_5D_5N$, 600 MHz) and $^{13}$C-NMR ($C_5D_5N$, 150 MHz) are given in Tables 1 and 2.

**Acid Hydrolysis of Compounds 1 and 2 and Determination of Absolute Configuration of Monosaccharides** Each compound (3 mg) was heated in 2 M HCl (5 ml) at 90 °C for 4 h. The reaction mixture was extracted with $CHCl_3$ (5 ml×3). The $CHCl_3$ extract was purified by chromatography on Sephadex LH-20 (2.0×100 cm). Comparing TLC with authentic samples, the aglycone was determined to be quillaic acid (**1a**, *Rf*: 0.25, $CHCl_3$–MeOH, 20 : 1). Each remaining aqueous layer was concentrated to dryness to give a residue and dissolved in pyridine (2 ml), and then L-cysteine methyl ester hydrochloride (2 mg) was added to the solution. The mixture was heated at 60 °C for 1 h, and trimethylchlorosilane (0.5 ml) was added, followed by heating at 60 °C for 30 min. Then, the solution was concentrated to dryness and dissolved in water (1 ml×3), followed by extraction with *n*-hexane (1 ml×3). The hexane extract was subjected to GC/MS analysis. The absolute configurations of the monosaccharides were confirmed to be D-fucose, L-rhamnose, D-xylose, D-glucuronic acid, D-galactose, and D-glucose by comparison of the retention times of monosaccharide derivatives with those of standard samples: D-fucose (12.85 min), L-rhamnose (12.67 min), D-xylose (11.89 min), D-glucuronic acid (14.13 min), D-galactose (14.32 min), and D-glucose (14.01 min), respectively.

Quillaic Acid (**1a**): White needles; ESI-MS $m/z$: 487 $[M+H]^+$; $^1$H-NMR (DMSO-$d_6$, 500 MHz) $\delta$: 0.77 (3H, s, Me), 0.92 (3H, s, Me), 0.95 (3H, s, Me), 0.97 (3H, s, Me), 0.99 (3H, s, Me), 1.43 (3H, s, Me), 3.75 (1H, m, 3-H), 4.40 (1H, br s, –OH), 4.80 (1H, br s, 16-H), 5.30 (1H, t, $J$=3.2 Hz, 12-H), 9.40 (1H, s, –CHO), 12.09 (1H, br s, –COOH). $^{13}$C-NMR (DMSO-$d_6$, 125 MHz) $\delta$: 37.9 (C-1), 26.2 (C-2), 70.6 (C-3), 55.3 (C-4), 46.6 (C-5), 20.4 (C-6), 31.6 (C-7), 39.4 (C-8), 47.5 (C-9), 35.6 (C-10), 24.3 (C-11), 121.2 (C-12), 144.2 (C-13), 40.0 (C-14), 35.3 (C-15), 73.1 (C-16), 46.2 (C-17), 41.3 (C-18), 46.6 (C-19), 30.4 (C-20), 34.8 (C-21), 32.0 (C-22), 207.3 (C-23), 9.1 (C-24), 15.5 (C-25), 17.0 (C-26), 26.7 (C-27), 178.4 (C-28), 33.1 (C-29), 23.0 (C-30).

**Acknowledgment** This research work was supported by the National Natural Science Foundation of China for Outstanding Young Scientists (No. 30525032).

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