Ypsilandrosides U-Y, five new steroidal saponins from *Ypsilandra thibetica*

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**Abstract**

Phytochemical reinvestigation on the whole plants of *Ypsilandra thibetica* obtained four new spirostanol glycosides, named ypsilandrosides U-X (1–4), and one new cholestanol glycoside, named ypsilandroside Y (5). Their structures have been established by extensive spectroscopic data and chemical methods. Among them, compound 4 is a rare spirostanol glycoside which possesses a novel 5(6 → 7) abeo-steroidal aglycone, while compound 1 is a first spirostanol bisdesmoside attached to C-3 and C-12, respectively, isolated from the genus *Ypsilandra*. The induced platelet aggregation activity of the isolates was tested.

**Keywords**: *Ypsilandra thibetica*, Melanthiaceae, Ypsilandrosides U-Y, Spirostanol saponins, Cholestanol saponins

**Graphical Abstract**

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1 *Introduction*

*Ypsilandra* (Melanthiaceae) is distributed in southwestern China and Myanmar, which contains 5 species according to the updated classification of the Angiosperm Phylogeny Group IV [1]. Among them, *Ypsilandra thibetica* has been used in folk medicine for treatment of scrofula, dysuria, edema, uterine bleeding, and traumatic hemorrhage in China by the local people [2, 3]. Our
previous investigations discovered twenty eight new steroidal glycosides including nineteen spirotanol saponins, two furostanol saponins, three cholestanol saponins, two pregnane glycosides, and two C22-steroidal lactone glycosides from this species [4–10], some of which showed cytotoxicity [4, 5], antifungal [4, 6], antibacterial [6], anti-HIV-1 activities [7], and so on. For further investigation on the chemical constituents of this herb, four new spirotanol saponins (1–4) and one new cholestanol saponin (5) (Fig. 1) were obtained and structurally characterized. The current paper reports the isolation, structural elucidation, and the induced platelet aggregation activity of these isolates.

2 Results and discussion

Compound 1 was isolated as an amorphous powder. Its molecular formula was determined as C44H70O17 by the positive-ion HRESI-MS at m/z 893.4500 [M + Na]+ (calcld. for C44H70O17Na, 893.4505) and 13C NMR data (Table 2). The 1H NMR spectrum of 1 (Table 1) showed four methyl proton signals at δH 0.89 (s, CH3-18), 0.91 (s, CH3-19), 1.38 (d, J = 7.0 Hz, CH3-21), and 0.67 (d, J = 5.4 Hz, CH3-27), one olefinic proton signal at δH 5.18 (o, H-6), while three anomeric protons at δH 5.64 (d, J = 3.2 Hz, H-1'), 5.30 (br s, H-1”), and 4.86 (d, J = 7.8 Hz, H-1”), which suggested that 1 was a glycoside with three monosaccharide moieties. The 13C NMR spectra displayed 44 carbon signals, of which 17 were assigned to those of one pentose and two hexose units, whereas other 27 ones were assigned to the aglycone moiety, including four methyl groups, nine methylene groups (one oxygenated), ten methine groups (one olefinic and three oxygenated), and four quaternary carbons (one olefinic and one ketal). The above NMR data suggested that compound 1 is a typical C-27 steroidal saponin and its aglycone is heloniogen [11]. This deduction can be confirmed by 2D-NMR spectra. The 1H–1H COSY correlations revealed that the aglycone for 1 had four structural fragments as shown in (Fig. 2). Furthermore, the key HMBC correlations (Fig. 2) from CH3-18 (δH 0.89) to C-12 (δC 82.4)/C-13 (δC 44.9)/C-14 (δC 44.4)/C-17 (δC 53.1), from CH3-19 (δH 0.91) to C-1 (δC 37.1)/C-5 (δC 141.1)/C-9 (δC 49.0)/C-10 (δC 36.9), from CH3-21 (δH 1.38)/H-20 (δH 2.00)/H-23a (δH 1.77)/H-26a (δH 3.53) to C-22 (δC 109.3) were observed. In addition, the ROESY correlations of H-12 (δH 3.88) with H-18 (δH 0.89) and H-20 (δH 2.00) indicated that the OH-12 was α-oriented (Fig. 3).

For the sugar part, the pentose was inferred as β-d-apiofuranoside by the 13C NMR signals at δC 108.1 (d, C-1), 78.4 (d, C-2), 79.0 (s, C-3), 74.8 (t, C-4), and 72.9 (t, C-5) with those of corresponding carbons of α- and β-d-apiofuranose and α- and β-l-apiofuranose [12, 13]. And the two hexose units were assigned to be a l-rhamnopyranosyl and a d-glucopyranosyl by their NMR data, the acid hydrolysis of 1, and the HPLC analysis (retention time) of their L-cysteine methyl esters followed by conversion into O-tolyl isothiocyanate derivatives and the authentic samples' derivatives. And the β-configuration of glucopyranosyl was revealed by the coupling constant (J1,2 > 7.0 Hz) [14], while the anomic
| Position | $1^a$ | $2^a$ | $3^a$ | $4^a$ | $5^b$ |
|----------|-------|-------|-------|-------|-------|
| 1a       | 1.73 (o) | 1.75 (d, 3.6) | 1.73 (o) | 1.70 (o) | 1.73 (m) |
| 1b       | 1.07 (d, 3.8) | 0.97 (s) | 1.00 (s) | 1.15 (o) | 0.95 (m) |
| 2a       | 1.99 (m) | 2.07 (o) | 2.21 (m) | 2.18 (m) | 2.06 (d) |
| 2b       | 1.62 (m) | 1.72 (d, 6.3) | 1.86 (m) | 1.89 (m) | 1.86 (d) |
| 3        | 3.58 (o) | 3.69 (m) | 3.95 (o) | 4.03 (m) | 3.87 (m) |
| 4a       | 2.45 (m) | 2.60 (o) | 2.72 (o) | 4.02 (m) | 2.81 (o) |
| 4b       | 2.29 (m) | 2.45 (t, 12.3) | 1.82 (o) | 2.47 (m) | 2.74 (o) |
| 6        | 5.18 (o) | 5.26 (o) | 5.22 (d, 5.0) | 10.22 (s) | 5.35 (o) |
| 7a       | 1.76 (o) | 1.91 (o) | 1.83 (m) | 1.95 (o) | |
| 7b       | 1.73 (o) | 1.60 (o) | 1.47 (m) | 1.58 (o) | |
| 8        | 1.44 (m) | 1.51 (m) | 1.49 (m) | 2.67 (m) | 1.61 (m) |
| 9        | 1.87 (d, 3.8) | 0.95 (d, 20.9) | 0.91 (m) | 1.03 (m) | 0.98 (m) |
| 11a      | 2.22 (m) | 1.56 (m) | 1.48 (m) | 1.32 (o) | 1.57 (2H, m) |
| 11b      | 1.57 (m) | 1.47 (m) | 1.03 (o) | |
| 12a      | 3.88 (br, s) | 2.18 (m) | 2.12 (m) | 1.69 (m) | 2.40 (m) |
| 12b      | 1.91 (m) | 1.83 (m) | 1.03 (m) | 1.60 (m) | |
| 14       | 1.56 (o) | 2.04 (m) | 2.03 (m) | 1.36 (m) | 1.50 (m) |
| 15a      | 1.94 (o) | 2.17 (m) | 2.21 (m) | 2.65 (m) | 2.61 (m) |
| 15b      | 1.44 (o) | 1.51 (m) | 1.56 (m) | 2.01 (m) | 2.41 (m) |
| 16       | 4.42 (m) | 4.43 (t, 6.9) | 4.58 (t, 7.2) | 4.59 (m) | |
| 17       | 3.31 (dd, 8.6, 6.1) | | | 1.80 (dd, 8.5, 6.1) | |
| 18       | 0.89 (s) | 0.93 (s) | 1.14 (s) | 0.88 (s) | 0.94 (s) |
| 19       | 0.91 (s) | 0.97 (s) | 1.00 (s) | 0.83 (s) | 1.10 (s) |
| 20       | 2.00 (m) | 2.20 (q) | 3.39 (q, 7.2) | 1.98 (m) | |
| 21       | 1.38 (d, 7.0) | 1.18 (d, 7.1) | 1.31 (d, 7.2) | 1.13 (d, 7.0) | 2.34 (s) |
| 23a      | 1.77 (o) | 1.92 (m) | 4.00 (m) | 1.67 (m) | 2.76 (m) |
| 23b      | 1.38 (o) | 1.52 (m) | 1.58 (m) | 2.72 (m) | |
| 24a      | 1.67 (m) | 2.21 (m) | 2.29 (o) | 1.58 (m) | 1.95 (m) |
| 24b      | 1.28 (m) | 1.86 (m) | 2.21 (o) | 1.24 (m) | 1.51 (m) |
| 25       | 1.55 (d, 6.2) | 1.87 (m) | 2.29 (m) | 1.59 (o) | 1.92 (m) |
| 26a      | 3.53 (o) | 4.12 (o) | 3.99 (m) | 4.90 (br s) | 3.79 (m) |
| 26b      | 3.46 (o) | 3.92 (m) | 3.90 (m) | 3.54 (br s) | 3.73 (m) |
| 27a      | 0.67 (d, 5.4) | 4.12 (o) | 3.73 (m) | 0.71 (d, 4.72) | 1.14 (d, 6.6) |
| 27b      | 3.94 (m) | 3.68 (m) | | | |
| 16’      | 3-Api | 3-Api | 3-Glc | 3-Glc | 7.12 (s) |
| 22’      | 3-Api | | | | 7.07 (s) |
| 1’       | 5.64 (d, 3.2) | 5.72 (o) | 4.92 (d, 7.1) | 5.02 (d, 7.3) | 4.96 (o) |
| 2’       | 4.63 (o) | 4.83 (m) | 4.18 (o) | 4.23 (m) | 4.22 (m) |
| 3’       | 4.18 (o) | 4.18 (o) | 4.20 (m) | 4.22 (m) | |
| 4a       | 4.51 (m) | 4.48 (d, 9.3) | 4.19 (o) | 4.40 (m) | 4.41 (m) |
| 4b       | 4.25 (m) | 4.24 (d, 9.3) | 3.89 (m) | 3.64 (m) | 3.61 (m) |
| 5a       | 4.62 (o) | 4.13 (2H, o) | | | |
| 5b       | 4.43 (o) | | | | |
| 6a       | 4.74 (d, 5.5) | | 4.21 (o) | 4.19 (o) | |
| 6b       | 4.28 (d, 5.5) | 4.05 (o) | 4.05 (o) | 4.05 (o) | |
| 5’-Rha   | 5’-Rha | 2’-Rha | 2’-Rha | | |
| 1*       | 5.30 (br s) | 5.85 (br s) | 6.31 (br s) | 6.44 (br s) | 6.41 (br s) |
| 2*       | 3.92 (m) | 4.56 (o) | 4.76 (o) | 4.86 (m) | 4.87 (m) |
| 3*       | 4.08 (m) | 4.70 (br, s) | 4.58 (o) | 4.62 (m) | 4.67 (m) |
configuration of rhamnopyranosyl was identified as \(\alpha\)-orientated on the basis of the chemical shift values of C-3' (\(\delta^C\) 72.9) and C-5' (\(\delta^C\) 70.6) with those of corresponding carbons of methyl \(\alpha\)- and \(\beta\)-rhamnopyranoside [15]. The sequence of the sugar chain at C-3 of the aglycone was established from the following HMBC correlations: H-1' (\(\delta^H\) 5.64) of Api with C-3 (\(\delta^C\) 77.5) of the aglycone, H-1'' (\(\delta^H\) 5.30) of the Rha with C-5'' (\(\delta^C\) 72.9) of Api, and H-1''' (\(\delta^H\) 4.86) of the Glc with C-12 (\(\delta^C\) 82.4) of the aglycone (Fig. 2). Thus, the structure of 1 was
Therefore, the structure of two monosaccharides and their absolute configurations [16] revealed that they shared the same aglycone. The absorption maxima at 254.5 nm, suggesting the presence of a conjugated enal system. When comparing its 1H and 13C NMR data (Tables 1 and 2) with those of ypsiparoside C obtained from the same genus [16] revealed that they shared the same aglycone. The two monosaccharides and their absolute configurations were determined as β-D-apiose and α-L-rhamnose by the same methods with compound 1. The HMBC correlations from H-1’ (δH 5.72) to C-3 (δC 77.7), and from H-1” (δH 5.85) of the rhamnopyranosyl to C-2’ (δC 82.4) established the sequence for 3-O-sugar chain as O-α-L-rhamnopyranosyl-(1 → 2)-β-D-apiofuranoside (Fig. 2). Therefore, the structure of 2 was determined as (25R)-spirost-5-en-3β,12β-diol-3-O-α-L-rhamnopyranosyl-(1 → 5)-β-D-apiofuranoside, and named ypsilandroside U.

Compound 2 was isolated as an amorphous powder with a molecular formula of C38H60O13 determined by the positive-ion HRESI-MS at m/z 747.3921 [M + Na]⁺, (calcd. for C38H60O13Na, 747.3926) and 13C NMR data (Table 2). Its NMR spectra suggested that 2 is a spirostane saponin with a disaccharide chain. Comparison of the 1H and 13C NMR data of 2 (Tables 1 and 2) with those of ypsiparoside C obtained from the same genus [16] revealed that they shared the same aglycone. The two monosaccharides and their absolute configurations were determined as β-D-apiose and α-L-rhamnose by the same methods with compound 1. The HMBC correlations from H-1’ (δH 5.72) to C-3 (δC 77.7), and from H-1” (δH 5.85) of the rhamnopyranosyl to C-2’ (δC 82.4) established the sequence for 3-O-sugar chain as O-α-L-rhamnopyranosyl-(1 → 2)-β-D-apiofuranoside (Fig. 2). Therefore, the structure of 2 was determined as (25R)-spirost-5-en-3β,17α,27-triol-3-O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside, and named ypsilandroside W.

Compound 4 possessed a molecular formula C51H80O21 determined by the HRESI-MS at m/z 1051.5077 [M + Na]⁺, (calcd. for C51H80O21Na, 1051.5084) and 13C NMR data (Table 2). The UV spectrum of 4 showed absorption maxima at 254.5 nm, suggesting the presence of a conjugated enal system. When comparing its 1H and 13C NMR data (Tables 1 and 2) with those of ypsilandroside H [10], it was suggested that they shared the same sugar sequence and the similar aglycone, except for the compound 4 has no hydroxyl substituent at the C-17. The above deduction could be verified by the HMBC correlations from H-21 (δH 1.13) and H-18 (δH 0.88) to C-17 (δC 62.4) and 1H-1H COSY correlations between H-16 (δH 4.59) and H-17 (δH 1.80) (Fig. 2). The HMBC correlations from H-1’ (δH 5.02) to C-3 (δC 77.7), from H-1” (δH 6.44) to C-2’ (δC 77.9), from H-1’’ (δH 5.82) to C-4’ (δC 77.7), and from H-1’’’ (δH 6.28) to C-4’’ (δC 80.4) confirmed that compound 3 had the same sequence of 3-O-sugar chain as that of ypsilandroside H (Fig. 2). Thus, the structure of 4 was elucidated as (25R)-B-nor(7)-6-carboxaldehyde-spirost-5(7)-en-3β-ol-3-O-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside, and named ypsilandroside V.

Compound 3 was isolated as an amorphous powder and had a molecular formula of C46H62O20 as determined by the positive-ion HRESI-MS data (m/z 955.4505 [M + Na]⁺, calcd. for C46H62O20Na, 955.4509) and 13C NMR data (Table 2). Inspection of the NMR spectra (Tables 1 and 2) of 3 revealed that it possessed a spirostanol skeleton with a trisaccharide chain consisting of one rhamnopyranosyl and two glucopyranosyls. Comparing its 1H and 13C NMR data (Tables 1 and 2) with those of trillitschonide S6 [17] indicated that they shared the same aglycone. The α-orientations of OH-23 and CH2OH-25 were supported by the ROESY correlations between H-23 (δH 4.00) and H-20 (δH 3.39)/H-25 (δH 2.29) (Fig. 3). The absolute configurations and the anomeric configurations of monosaccharides were determined by the same methods with the above compounds. The sequence of the sugar chain at C-3 of the aglycone was established by the HMBC correlations from H-1’ (δH 4.92) to C-3 (δC 76.8), from H-1” (δH 1.13) to C-2’ (δC 77.5), and from H-1’’ (δH 5.04) to C-6’ (δC 69.9) (Fig. 2). Consequently, the structure of 3 was elucidated as (23S,25S)-spirost-5-en-3β,17α,23,27-tetraol-3-O-β-D-glucopyranosyl-(1 → 6)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside, and named ypsilandroside W.

The molecular formula of compound 5 was determined as C53H82O19 by the HRESI-MS at m/z 1045.5352 [M + Na]⁺, (calcd. for C53H82O19Na, 1045.5343) and 13C NMR data (Table 2). Its NMR spectra indicated that compound 5 was a cholestane tetracyglcosides containing an aromatic ring. Analysis of the 1H and 13C NMR data (Tables 1 and 2) of 5 suggested that it was similar to that of parispseudoside A [18], and the major difference was the absence of a glucopyranosyl group at OH-26 site. With the assistance of HSQC experiment, 1H and 13C NMR data (Tables 1 and 2) showed
Table 2

| Position | 1<sup>a</sup> | 2<sup>a</sup> | 3<sup>a</sup> | 4<sup>a</sup> | 5<sup>b</sup> |
|----------|----------------|----------------|----------------|----------------|----------------|
| 6<sup>″″</sup> | 63.1 (t) | 62.7 (t) | 18.6 (q) | 18.8 (q) | 4<sup>″″</sup>-Rha 4<sup>″″</sup>-Rha |
| 1<sup>″″</sup> | 103.4 (d) | 103.2 (d) | 72.7 (d) | 72.8 (d) | 3<sup>″″</sup>-Rha 3<sup>″″</sup>-Rha |
| 2<sup>″″</sup> | 72.9 (d) | 72.4 (d) | 74.0 (d) | 74.1 (d) | 5<sup>″″</sup>-Rha 5<sup>″″</sup>-Rha |
| 3<sup>″″</sup> | 70.5 (d) | 70.3 (d) | 18.9 (q) | 18.6 (q) | 4<sup>″″</sup>-Rha 4<sup>″″</sup>-Rha |

* Measured at 125 MHz, * Measured at 150 MHz

four anomic protons at δ<sub>H</sub> 4.96 (o, H-1′), 6.41 (br s, H-1′), 5.84 (br s, H-1″), and 6.29 (s, H-1‴) and their corresponding anomic carbons at δ<sub>"C"</sub> 100.2 (C-1′), 102.1 (C-1″), 102.1 (C-1‴), and 103.2 (C-1″). The sequence of sugar units was consistent with that of compound 4 by HMBC experiment (Fig. 2). As a result, the structure of 5 was assigned as homo-ar-o-cholest-5-en-3β,26-diol-3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, and named ypsilandside Y.

Because the whole plants of *Y. thibetica* have been used in folk medicine for treatment of uterine bleeding and traumatic hemorrhage in China, the isolated compounds (1–5) were evaluated for their induced platelet aggregation activity and ADP (adenosine diphosphate) was used as a positive control. Unfortunately, the results showed all isolated saponins did not exhibit the inducing platelet aggregation activity at the tested concentration of 100 μM.

### 3 Experimental section

#### 3.1 General experimental procedures

Optical rotations were measured by a JASCO P-1020 polarimeter (Jasco Corp., Japan). UV spectra were recorded on a Shimadzu UV2401 PC spectrophotometer (Shimadzu Corp., Japan). HRESI-MS was recorded on an Agilent 1290 UPLC/6540 Q-TOF mass spectrometer (Agilent Corp., USA). The NMR experiments were performed on Bruker AVANCE III 500, Avance III-600, and AV 800 spectrometers (Bruker Corp., Switzerland). Silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd., People's Republic of China), RP-18 (50 μm, Merck, Germany), and Sephadex LH-20 (Pharmacia, Stockholm, Sweden) were used for column chromatography (CC). An Agilent 1260 system (Agilent Corp., America) with a Zorbax SB-C18 column (5 μm, 9.4 × 250 mm) was used for HPLC separation. TLC was carried out on silica gel
HSGF₃₅₄ plates (Qingdao Marine Chemical Co., China) or RP-18 F₂₅₄ (Merck, Darmstadt, Germany).

3.2 Plant material
The whole plant materials of *Y. thibetica* were collected in August 2010 from Zhaotong City, Yunnan Province, China, and identified by Prof. Xin-Qi Chen, Institute of Botany, Chinese Academy of Sciences, Beijing. A voucher specimen was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation
The dried whole plants of *Y. thibetica* (110 kg) were crushed and extracted three times with 70% EtOH under reflux for 3 h, 2 h and 2 h. Then, the combined extract was concentrated under reduced pressure. The crude extract (30 kg) was passed through YWD-3F macroporous resin and eluted successively with H₂O, 40% EtOH, 75% EtOH, and 95% EtOH, respectively. Evaporated 75% EtOH fraction (crude saponin-rich mixture, 10 kg) was subjected to a silica gel column chromatography (CHCl₃–MeOH) to give three subfractions (C-13–1–C-13–3). Subsequently, Fr. C-13–1 (150 mg) was further purified by preparative HPLC (MeCN–H₂O, 30:70, v/v) to give eleven fractions (Fr. A–Fr. C-13–1–Fr. C-13–3). Fr. C-13 (45 g) was submitted to Sephadex LH-20 (MeOH) and chromatographically separated on an RP-18 column eluted with MeOH–H₂O (40:60→70:30, v/v) and purified by preparative HPLC (MeCN–H₂O, 40:60→50:50, v/v) to afford saponins 2 (tᵣ=12.8 min, 10 mg). Fr. C-13 (45 g) was submitted to Sephadex LH-20 (MeOH) to give three subfractions (C-13–1–C-13–3). Subsequently, Fr. C-13–1 (150 mg) was further purified by preparative HPLC (MeCN–H₂O, 25:75→35:65, v/v) to afford saponins 5 (tᵣ=10.8 min, 7 mg) and 4 (tᵣ=11.9 min, 12 mg), whereas saponins 3 (tᵣ=11.1 min, 10 mg) and 1 (tᵣ=14.8 min, 9 mg) were obtained from Fr. C-13–3 (208 mg) by preparative HPLC (MeCN–H₂O, 30:70→45:55, v/v).

3.4 Physical and spectroscopic data of new glycosides

3.4.1 *Ypsilandroside U* (1)
Amorphous solid; [α]D²⁵ -55.80 (c 0.20, MeOH); ¹H (500 MHz, pyridine-d₅) and ¹³C (125 MHz, pyridine-d₅) NMR data, see Tables 1 and 2; HRESIMS m/z 893.4500 [M+Na]⁺ (calcd. for C₅₃H₇₂O₉Na, 893.4505) (Additional file 1).

3.4.2 *Ypsilandroside V* (2)
Amorphous solid; [α]D²⁵ -190.00 (c 0.12, MeOH); ¹H (500 MHz, pyridine-d₅) and ¹³C (125 MHz, pyridine-d₅) NMR data, see Tables 1 and 2; HRESIMS m/z 747.3921 ([M+Na]⁺, calcd. for C₃₉H₆₀O₁₃Na, 747.3926) (Additional file 1).

3.4.3 *Ypsilandroside W* (3)
Amorphous solid; [α]D²⁶ -125.67 (c 0.12, MeOH); ¹H (500 MHz, pyridine-d₅) and ¹³C (125 MHz, pyridine-d₅) NMR data, see Tables 1 and 2; HRESIMS m/z 955.4505 [M+Na]⁺ (calcd. for C₄₅H₇₂O₂₀Na, 955.4509) (Additional file 1).

3.4.4 *Ypsilandroside X* (4)
Amorphous solid; [α]D²⁶ -48.18 (c 0.11, MeOH); UV (MeOH) λ max (log ε) 203 (4.5) nm; ¹H (600 MHz, pyridine-d₅) and ¹³C (150 MHz, pyridine-d₅) NMR data, see Tables 1 and 2; HRESIMS m/z 1045.5352 [M+Na]⁺ (calcd. for C₅₃H₇₂O₂₁Na, 1051.5084) (Additional file 1).

3.4.5 *Ypsilandroside Y* (5)
Amorphous solid; [α]D²⁶ -48.18 (c 0.11, MeOH); UV (MeOH) λ max (log ε) 203 (4.5) nm; ¹H (600 MHz, pyridine-d₅) and ¹³C (150 MHz, pyridine-d₅) NMR data, see Tables 1 and 2; HRESIMS m/z 1045.5352 [M+Na]⁺ (calcd. for C₅₃H₇₂O₂₁Na, 1051.5084) (Additional file 1).

3.5 Acid hydrolysis of compounds 1–5 and determination of the absolute configuration of the sugars by HPLC
Compounds 1–5 (1.0 mg each) in 6 M CF₃COOH (1,4-dioxane–H₂O 1:1, 1.0 mL) were heated at 99 °C for 2 h, respectively. The reaction mixture was diluted with H₂O (1.0 mL) and then extracted with EtOAc (3×2.0 mL). Next, each aqueous layer was evaporated to dryness using rotary evaporation. Each dried residue was dissolved in pyridine (1.0 mL) mixed with l-cysteine methyl ester hydrochloride (1.0 mg) (Aldrich, Japan) and heated at 60 °C for 1 h. Then, O-tolyl isothiocyanate (5.0 μL) (Tokyo Chemical Industry Co., Ltd., Japan) was added to the mixture, this being heated at 60 °C for 1 h. Each reaction mixture was directly analyzed by reversed phase HPLC following the above procedure. Each reaction mixture was directly analyzed by analytical HPLC on a Poroshell 120 SB-C18 column (100×4.6 mm, 2.7 μm, Agilent) using an elution of CH₃CN–H₂O (20:75→40:60, v/v) at a flow rate of 0.6 mL/min. As a result, the sugars in the test compounds were identified as d-glucose and l-rhamnose, respectively, by comparing their molecular weight and retention time with the standards (tᵣ 13.90 min for d-glucose; tᵣ 17.72 min for l-rhamnose).

3.6 Platelet aggregation assays
Turbidometric measurements of platelet aggregation of the samples were performed in a Chronolog Model 700 Aggregometer (Chronolog Corporation, Havertown,
PA, USA) according to Born’s method [19, 20]. Rabbit platelet aggregation study was completed within 3.0 h of preparation of platelet-rich plasma (PRP). Immediately after preparation of PRP, 250 μL was incubated in each test tube at 37 °C for 5.0 min and then 2.5 μL of compounds (100 μM) were individually added. The changes in absorbance as a result of platelet aggregation were recorded. The extent of aggregation was estimated by the percentage of maximum increase in light transmittance, with the buffer representing 100% transmittance. ADP (adenosine diphosphate) was used as a positive control with a 59.5 ± 6.1% maximal platelet aggregation rate at a concentration of 10 μM. 1% DMSO was used as a blank species.

Ypsilandra after preparation of PRP, 250 μL was incubated in each preparation of platelet-rich plasma (PRP). Immediately after incubation, ADP was added as a positive control. The extent of aggregation was estimated by the percentage of maximum increase in light transmittance, with the buffer representing 100% transmittance. ADP (adenosine diphosphate) was used as a positive control with a 59.5 ± 6.1% maximal platelet aggregation rate at a concentration of 10 μM. 1% DMSO was used as a blank species.

4 Conclusion
Phytochemical reinvestigation on the whole plants of Y. thibetica obtained four new spirostanol glycosides, named ypsilandrosides U-X (1–4), and one new cholestanol glycoside, named ypsilandroside Y (5). Their structures have been illustrated by extensive spectroscopic data and chemical methods. Among them, compound 4 is a rare spirostanol glycoside which possesses a novel 5(6 → 7) abeo-steroidal aglycone, while compound 1 is a first spirostanol bisdesmoside attached to C-3 and C-12, respectively, obtained from the Ypsilandra species. This investigation enriched the cognition of the chemical constituents in Y. thibetica. Unfortunately, the bioassay results showed the five new saponins have no the activity of inducing platelet aggregation.

Supplementary Information
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Author contributions
All authors read and approved the final manuscript.

Declarations
Competing interests
The authors declare that there are no conflicts of interest associated with this work.

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