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Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

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To cite this article: Thi Kieu My Than, Thanh Ky Pham, Thanh Binh Pham, Thao Nguyen Phuong & Dat Nguyen Tien (2018): New dammarane-type triterpenoid glycosides from *Gynostemma burmanicum*, Natural Product Research, DOI: 10.1080/14786419.2018.1527831

To link to this article: https://doi.org/10.1080/14786419.2018.1527831

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2018.1527831

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RESEARCH-ARTICLE

New dammarane-type triterpenoid glycosides from *Gynostemma burmanicum*

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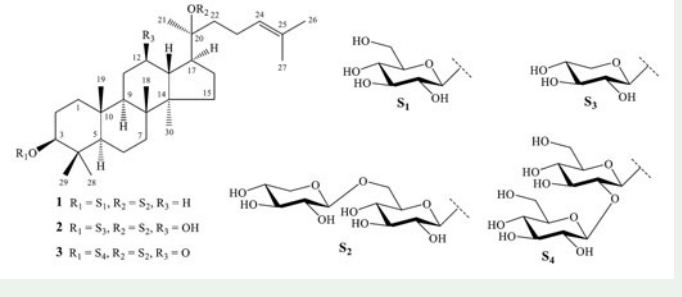
ABSTRACT

The chemical composition of *Gynostemma burmanicum* King ex Chakrav. was investigated for the first time in this study. Nine dammarane glycosides (**1**–**9**) were isolated from the EtOH extract of the aerial parts of *G. burmanicum*. Their structures were elucidated by 1D and 2D NMR spectroscopic interpretation as well as by chemical studies. The new compounds were 3β,20*S*-dihydroxydammar-24-ene-3-O-β-D-glucopyranosyl-20-O-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] (**1**), 3β,12β,20*S*-trihydroxydammar-24-ene-3-O-β-D-xylopyranosyl-20-O-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] (**2**), and 12-oxo-3β,20*S*-dihydroxydammar-24-ene-3-O-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-O-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] (**3**).



ARTICLE HISTORY
Received 19 June 2018
Accepted 20 September 2018

KEYWORDS
Gynostemma burmanicum; Cucurbitaceae; dammarane saponin

1. Introduction

The genus *Gynostemma* Blume (Cucurbitaceae) composes of approximately 16 species, distributed in forests, scrubs and bush habitats at 60–3200 m elevations throughout China, India, Vietnam, Myanmar, Korea, and Japan (Chen 1995). They have been used in folk medicine to lower cholesterol levels, regulate blood pressure, strengthen the

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2018.1527831

immune system, and reduce inflammation (Li et al. 1993). In recent years, several studies on *Gynostemma* species have been carried out, including saponins, flavonoids, amino acids, and reducing sugars, which could be commercially useful. Dammarane-type saponins are important metabolites of *Gynostemma* plants, which have been shown to possess various biological activities as neuroprotective (Ky et al. 2010; Seo et al. 2017), antioxidant (Seo et al. 2017), inflammatory (Wong et al. 2017; Han et al. 2018), and osteoporosis (Han et al. 2018; Razmovski-Naumovski et al. 2005) activities. Among *Gynostemma* plants, *G. pentaphyllum* has been most studied (Li et al. 2016; Razmovski-Naumovski et al. 2005). *Gynostemma burmanicum* King ex Chakrav. is widely distributed in South-East Asia, South China, Bangladesh (POWO 2018), and has not been investigated for their chemical composition. In the present paper, we described the structural isolation and elucidation of three new saponins (**1**−**3**), along with 6 known dammarane-type saponins (**4**–**9**, Figure 1).

2. Results and discussion

Compound **1** was isolated as a white, amorphous powder. The molecular formula, $C_{47}H_{80}O_{16}$, was deduced from its HR-ESI-MS data (*m/z* 899.5334 $[M - H]^-$, calcd. for $C_{47}H_{79}O_{16}$, 899.5338) and ^{13}C NMR data (see Experimental section). The ^{13}C NMR and DEPT spectra showed 47 carbon resonances, of which 30 were assigned to a aglycone 3β,20*S*-dihydroxydammar-24-en (Takemoto et al. 1983) and 17 to the sugar moieties. The ^1H NMR spectrum of **1** showed eight singlets assignable to the aglycone methyls [δ_H 1.01 (H-18), 0.91 (H-19), (H-26), 1.63 (H-27), 1.06 (H-28), 0.86 (H-29), and 0.93 (H-30), each 3H], two of which were diagnostic for methyls linked to a sp^2 carbon [δ_H 1.70

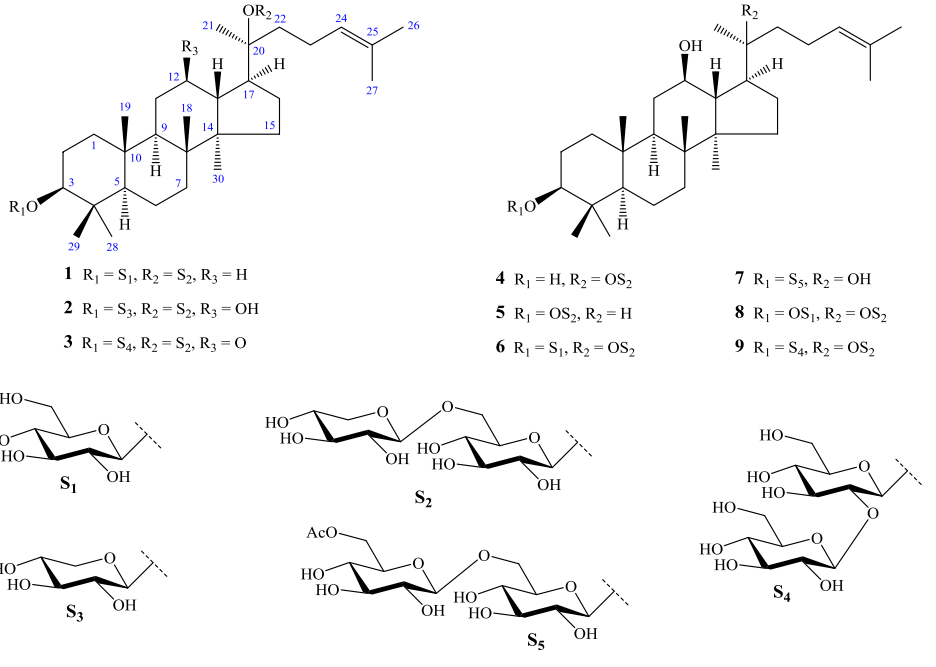

Figure 1. Structures of compounds (**1**−**9**) isolated from *G. burmanicum*.

(C-26) and 1.63 (C-27)], an olefinic proton signal [δ_H 5.11 (1H, t, $J = 6.0$ Hz, H-24)]. Additionally, signals for three anomeric protons [δ_H 4.43 (1H, d, $J = 7.5$ Hz, H-1′), 4.49 (1H, d, $J = 7.5$ Hz, H-1″), and 4.30 (1H, d, $J = 7.5$ Hz, H-1‴) were observed in the ^{1}H NMR spectrum, suggesting the presence of three sugar units in its structure. In comparison with the corresponding signals in a model compound reported in the literature (Takemoto et al. 1983), glycosidations at C-3 and C-20 were indicated by the significant downfield shift observed for these carbon signals in **1**. Acid hydrolysis of **1** yielded D-glucose and D-xylose (2:1), which were confirmed by GC/MS analysis (Thao et al. 2016). The chemical shifts, the signal multiplicities, coupling constant values, and their magnitude in the ^{1}H NMR spectrum, as well as the ^{13}C NMR data, indicated that both sugar units have a β-configuration, which were confirmed by the analysis of their HMBC and ^{1}H-^{1}H COSY spectra. The significant downfield shift of C-6″ (δ_C 70.1) in the inner β-glucopyranosyl moiety at C-20 of aglycone in the ^{13}C NMR spectrum together with the HMBC correlation between H-1‴ [δ_H 4.30 (d, $J = 7.5$ Hz)] with C-6″ (δ_C 70.1) showed that the terminal β-xylopyranosyl is linked to the inner C-20 by a specific 1→6 linkage (see Supporting data). These linkages were further confirmed by NOESY correlations between H-1′/H-3 and H-1‴/H-6″.

The relative configuration of **1** was determined from the NOESY experiments and from the *J* values, which showed NOESY cross-peaks (H-3α/Me-28, H-3α/H-5α, Me-29/Me-19, Me-19/Me-18, H-30/H-17, and H-17/Me-21) and a large coupling constant of H-3 ($J = 9.5$ Hz). These data permitted the H-3 and C-20 to be established as α and *S*, respectively. On the basis of the above results, the structure of **1** was determined as 3β,20*S*-dihydroxydammar-24-ene-3-O-β-D-glucopyranosyl-20-O-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside].

Compound **2** was also isolated as a white, amorphous powder. The molecular formula of $C_{46}H_{78}O_{16}$ was assigned from the HR-ESI-MS data (*m/z* 885.5215 $[M-H]^-$, calcd. for $C_{46}H_{77}O_{16}$, 885.5211). The ^{1}H and ^{13}C NMR data were also closely comparable to those of gypenoside IX (**8**) for the aglycone moiety (Takemoto et al. 1983); however, the major differences were observed in that the sugar moiety. This difference was attributed to the attachment of the sugar moiety at C-3, suggesting that the aglycone of **2** was identical to that of **8**. These results, including the HMBC and COSY data, indicated that the glucose unit of **8** was replaced by a xylose moiety in **2**. Furthermore, the location of the xylose moiety was determined as at the C-3 position by the HMBC correlation between H-1′ (δ_H 4.24) with C-3 (δ_C 90.8). The locations of these remaining disaccharides were determined as at the C-20 position by the observed HMBC correlations between H-1″ (δ_H 4.55)/C-20 (δ_C 83.8) and H-1‴ (δ_H 4.28)/C-6″ (δ_C 70.0) in the same manner as for **1** (see Supporting data). (Takemoto et al. 1983). Moreover, acid hydrolysis of **2** gave D-xylose and D-glucose in a ratio of 2:1 by GC/MS analysis (Thao et al. 2016).

The configuration of **2** was determined from the NOESY experiments and from the *J* values. The relative configuration of **2** was elucidated as H-3α, H-12β, and 20*S* in the same manner as for **1** and **8** (Takemoto et al. 1983). The α-configuration for H-3 was determined in the same manner as for **1** on the basis of ROESY correlations between H-3α/Me-28 and H-3α/H-5α, whereas another correlation between Me-29/Me-19, Me-19/Me-18, H-12α/H-30, H-30/H-17α, and H-17α/Me-21 confirmed the β-configuration

for the substituent OH-12 and *S*-configuration at C-20 (see Supporting data). Accordingly, compound **2** was determined as 3β,12β,20*S*-trihydroxydammar-24-ene-3-O-β-D-xylopyranosyl-20-O-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside].

Compound **3** was obtained as a white, amorphous powder. Its molecular formula of $C_{53}H_{88}O_{22}$ was determined by HR-ESI-MS (*m/z* 1075.5663 $[M-H]^-$, calcd. for $C_{53}H_{87}O_{22}$, 1075.5668). The 1D and 2D NMR spectrum of **3** suggested a dammarane-type triterpenoid skeleton with a carbonyl function. By comparison of its NMR spectra with those of ginsenoside Rb3 (**9**) showed the absence of a 12-oxymethine signal, and an additional quaternary carbonyl carbon signal (δ_C 215.5) was observed (Sanada and Shoji 1978). Analysis of the ^{13}C NMR data of **3**, the carbonyl group was placed at C-12. This assignment was supported by the HMBC correlations between H-13 (δ_H 3.32) and H-11a (δ_H 2.13)/H-11b (δ_H 2.43) with the quaternary carbonyl signal (δ_C 215.5, C-12). Hydrolysis of compound **3** yielded D-xylose and D-glucose. By GC analysis of the acetate derivatives of the component monosaccharides, it was clear that **3** contained three units of D-glucose and one of D-xylose. The location of the each sugar moiety was determined by an HMBC experiment [δ_H 4.44 (H-1′)/δ_C 90.9 (C-3), δ_H 4.69 (H-1″)/δ_C 81.2 (C-2′), δ_H 4.46 (H-1‴)/δ_C 82.6 (C-20), and δ_H 4.31 (H-1⁗)/δ_C 70.0 (C-6‴), indicating that the four sugar moieties (two glucose and a xylosyl-glucose) could be located at C-3 and C-20, respectively (see Supporting data). The configuration of **3** was elucidated as H-3α and 20*S* in the same manner as for **1** and **2**. Thus, compound **3** was elucidated as 12-oxo-3β,20*S*-dihydroxydammar-24-ene-3-O-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-O-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside].

Together with three new saponins (**1**−**3**), the 6 known dammarane-type saponins were identified by comparison of their reported spectroscopic data as gypenoside XIII (**4**) (Takemoto et al. 1983), ginsenoside Rg5 (**5**) (Yang et al. 2014), ginsenoside F2 (**6**) (Yahara et al. 1976), ginsenoside Rg3 (**7**) (Yang et al. 1983), gypenoside IX (**8**) (Takemoto et al. 1983), and ginsenoside Rb3 (**9**) (Sanada and Shoji 1978) (Figure 1).

3. Experimental

3.1. General procedures

Thin layer chromatography (TLC) was performed using precoated Kiesel gel 60 F_{254} (Merck) and visualized by UV light 254 nm and 10% H_2SO_4 reagent by heating. Column chromatography (CC) was performed using silica gel 60 (Merck, 70–230 mesh) and C_{18} reverse phase powder (ODS-A 75 μm, YMC, Japan). NMR experiments were carried out on a Bruker AM500 FT-NMR spectrometer (Bruker, Rheinstetten, Germany) using residual solvent peak as a reference or tetramethylsilane (TMS) as internal standard. The HR-ESI-MS were recorded on a Waters Q-TOF micromass spectrometer Waters Q-TOF micromass spectrometer.

3.2. Plant materials

The aerial parts of *G. burmanicum* were collected in April 2015 at Bac Kan province, Vietnam and identified by Prof. Tran Van On, Hanoi University of Pharmacy. The

voucher specimens (No HNIP/18136/15) were deposited at the Herbarium of Hanoi University of Pharmacy.

3.3. Extraction and isolation

The air-dried and powdered aerial parts of *G. burmanicum* (3.5 kg) were extracted with EtOH (24 L × 3) at room temperature, and then the solution was evaporated in vacuo. The residue (650 g) was suspended in distilled water and partitioned with *n*-hexane and EtOAc to obtain *n*-hexane (15.7 g) and (74.8 g) EtOAc residues, respectively. The water fraction was chromatographed on a Diaion HP-20 column eluted with a step gradient of H_2O-MeOH (20:80 to 0:100) to give four fractions (GPB1-GPB4). Compounds **4** (57.0 mg), **5** (25.4 mg), **6** (7.0 mg), and **7** (5.7 mg) were purified from fraction GPB2 (7.5 g) after subjecting it to YMC RP-C_{18} CC eluted with acetone–H_2O (1.7:1, v/v), followed by silica gel CC using CH_2Cl_2–MeOH–H_2O (4.5:1:0.2, v/v/v). In a similar process to that describe above, fraction GPB3 (3.9 g) was further separated on silica gel CC with CH_2Cl_2–MeOH–H_2O (3:1:0.2, v/v/v) as eluent, followed by YMC RP-C_{18} CC with MeOH–H_2O (1:3, v/v) to obtain compounds **1** (8.4 mg), **2** (52.4 mg), and **8** (77 mg). Finally, compounds **3** (52.4 mg) and **9** (37.0 mg) were isolated from fraction GPB4 (3.1 g) by YMC RP-C_{18} CC eluted with acetone–H_2O (1:1.5, v/v).

3.3.1. 3β,20S-dihydroxydammar-24-ene-3-O-β-D-glucopyranosyl-20-O-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] (1)

White, amorphous powder. HR-ESI-MS *m/z* 899.5334 $[M - H]^-$ (Calcd. for $C_{47}H_{79}O_{16}$, 899.5338). ^{1}H NMR (CD_3OD, 500 MHz): δ_H 1.71 (1H, m, H-1a), 1.00 (1H, m, H-1b), 1.95 (1H, m, H-2a), 1.72 (1H, m, H-2b), 3.18 (1H, d, $J = 9.5$ Hz, H-3), 0.80 (1H, d, $J = 11.0$ Hz, H-5), 1.56 (1H, m, H-6a), 1.49 (1H, m, H-6b), 1.58 (1H, m, H-7a), 1.30 (1H, m, H-7b), 1.40 (1H, m, H-9), 1.53 (1H, m, H-11a), 1.29 (1H, m, H-11b), 1.74 (1H, m, H-12), 1.75 (1H, m, H-13), 1.49 (1H, m, H-15a), 1.04 (1H, m, H-15b), 1.85 (1H, m, H-16a), 1.34 (1H, m, H-16b), 1.97 (1H, m, H-17), 1.01 (3H, s, H-18), 0.91 (3H, s, H-19), 1.25 (3H, s, H-21), 1.58 (1H, m, H-22a), 1.30 (1H, m, H-22b), 2.07 (2H, m, H-23), 5.11 (1H, t, $J = 7.0$ Hz, H-24), 1.70 (3H, s, H-26), 1.63 (3H, s, H-27), 1.06 (3H, s, H-28), 0.86 (3H, s, H-29), 0.93 (3H, s, H-30); *C-3-Glc-1 (inner)*: δ_H 4.43 (1H, d, $J = 7.5$ Hz, H-1′), 3.30 (1H, dd, $J = 7.5$, 9.0 Hz, H-2′), 3.26 (1H, m, H-3′), 3.31 (1H, m, H-4′), 3.31 (1H, m, H-5′), 3.85 (1H, dd, $J = 2.5$, 11.0 Hz, H-6′a), 3.67 (1H, br d, $J = 11.0$ Hz, H-6′b); *C-20-Glc-1 (inner)*: δ_H 4.49 (1H, d, $J = 7.5$, H-1″), 3.32 (1H, m, H-2″), 3.36 (1H, m, H-3″), 3.38 (1H, m, H-4″), 3.38 (1H, m, H-5″), 4.00 (1H, d, $J = 2.5$, 11.0 Hz, H-6″a), 3.76 (1H, dd, $J = 5.0$, 11.0 Hz, H-6″b); *Xyl-1 (term)*: δ_H 4.30 (1H, d, $J = 7.5$ Hz, H-1‴), 3.23 (1H, m, H-2‴), 3.36 (1H, m, H-3‴), 3.50 (1H, m, H-4‴), 3.87 (1H, m, H-5‴a), and 3.21 (1H, t, $J = 10.5$ Hz, H-5‴b). ^{13}C NMR (CD_3OD, 125 MHz): δ_C 40.3 (C-1), 27.2 (C-2), 90.8 C-3), 40.4 (C-4), 57.7 (C-5), 19.2 (C-6), 36.5 (C-7), 41.6 (C-8), 52.1 (C-9), 38.0 (C-10), 22.7 (C-11), 26.0 (C-12), 43.5 (C-13), 51.5 (C-14), 32.2 (C-15), 28.8 (C-16), 48.9 (C-17), 16.1 (C-18), 16.9 (C-19), 83.8 (C-20), 21.5 (C-21), 40.8 (C-22), 23.6 (C-23), 126.3 (C-24), 131.7 (C-25), 25.9 (C-26), 18.0 (C-27), 28.4 (C-28), 16.8 (C-29), 17.1 (C-30); *C-3-Glc-1 (inner)*: δ_C 106.6 (C-1′), 75.6 (C-2′), 77.6 (C-3′), 71.6 (C-4′), 77.4 (C-5′), 62.8 (C-6′); *C-20-Glc-1 (inner)*: δ_C 98.6 (C-1″), 75.5 (C-2″), 78.4 (C-3″), 71.5

(C-4″), 76.2 (C-5″), 70.1 (C-6″); *Xyl-1 (term)*: δ_C 105.5 (C-1‴), 74.7 (C-2‴), 78.2 (C-3‴), 71.1 (C-4‴), and 66.7 (C-5‴).

3.3.2. 3β,12β,20S-trihydroxydammar-24-ene-3-O-β-D-xylopyranosyl-20-O-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] (2)

White, amorphous powder. HR-ESI-MS *m/z* 885.5215 $[M-H]^-$ (Calcd. for $C_{46}H_{77}O_{16}$, 885.5211). ^{1}H NMR (CD_3OD, 500 MHz): δ_H 1.70 (1H, m, H-1a), 0.97 (1H, m, H-1b), 1.80 (1H, m, H-2a), 1.68 (1H, m, H-2b), 3.10 (1H, d, $J = 9.5$ Hz, H-3), 0.76 (1H, d, $J = 11.0$ Hz, H-5), 1.54 (1H, m, H-6a), 1.46 (1H, m, H-6b), 1.54 (1H, m, H-7a), 1.28 (1H, m, H-7b), 1.41 (1H, m, H-9), 1.77 (1H, m, H-11a), 1.27 (1H, m, H-11b), 3.70 (1H, m, H-12), 1.71 (1H, m, H-13), 1.55 (1H, m, H-15a), 1.02 (1H, m, H-15b), 1.79 (1H, m, H-16a), 1.33 (1H, m, H-16b), 2.26 (1H, m, H-17), 0.98 (3H, s, H-18), 0.90 (3H, s, H-19), 1.34 (3H, s, H-21), 1.77 (1H, m, H-22a), 1.50 (1H, m, H-22b), 2.14 (1H, m, H-23a), 2.02 (1H, m, H-23b), 5.11 (1H, t, $J = 7.0$ Hz, H-24), 1.67 (3H, s, H-26), 1.61 (3H, s, H-27), 1.02 (3H, s, H-28), 0.83 (3H, s, H-29), 0.90 (3H, s, H-30); *C-3-Xyl-1 (inner)*: δ_H 4.24 (1H, d, $J = 7.5$ Hz, H-1′), 3.16 (1H, m, H-2′), 3.27 (1H, m, H-3′), 3.47 (1H, m, H-4′), 3.16 (2H, t, $J = 10.5$ Hz, H-5′); *C-20-Glc-1 (inner)*: δ_H 4.55 (1H, d, $J = 7.5$ Hz, H-1″), 3.11 (1H, m, H-2″), 3.33 (1H, m, H-3″), 3.31 (1H, m, H-4″), 3.38 (1H, m, H-5″), 4.00 (1H, dd, $J = 2.5$, 11.0 Hz, H-6″a), 3.73 (1H, br d, $J = 11.0$ Hz, H-6″b); *Xyl-1 (term)*: δ_H 4.28 (1H, d, $J = 7.5$ Hz, H-1‴), 3.19 (1H, m, H-2‴), 3.29 (1H, m, H-3‴), 3.44 (1H, m, H-4‴), 3.83 (1H, m, H-5‴a), and 3.18 (1H, t, $J = 10.5$ Hz, H-5‴b). ^{13}C NMR (CD_3OD, 125 MHz): δ_C 40.2 (C-1), 27.2 (C-2), 90.4 (C-3), 40.3 (C-4), 57.5 (C-5), 19.2 (C-6), 35.8 (C-7), 40.9 (C-8), 51.1 (C-9), 37.9 (C-10), 30.8 (C-11), 71.6 (C-12), 49.7 (C-13), 52.4 (C-14), 31.5 (C-15), 27.3 (C-16), 52.9 (C-17), 16.3 (C-18), 16.7 (C-19), 84.9 (C-20), 22.4 (C-21), 36.7 (C-22), 23.8 (C-23), 126.0 (C-24), 132.1 (C-25), 25.9 (C-26), 18.0 (C-27), 28.4 (C-28), 16.8 (C-29), 17.4 (C-30); *C-3-Xyl-1 (inner)*: δ_C 107.3 (C-1′), 75.4 (C-2′), 77.9 (C-3′), 71.2 (C-4′), 66.7 (C-5′); *C-20-Glc-1 (inner)*: δ_C 98.0 (C-1″), 75.2 (C-2″), 78.5 (C-3″), 71.4 (C-4″), 76.6 (C-5″), 70.0 (C-6″); *Xyl-1 (term)*: δ_C 105.4 (C-1‴), 74.7 (C-2‴), 77.4 (C-3‴), 71.1 (C-4‴), and 66.6 (C-5‴).

3.3.3. 12-oxo-3β,20S-dihydroxydammar-24-ene-3-O-[β-D-glucopyranosyl(1→2)-β-D-glucopyrano syl]-20-O-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside] (3)

White, amorphous powder. HR-ESI-MS *m/z* 1075.5663 $[M-H]^-$ (Calcd. for $C_{53}H_{87}O_{22}$, 1075.5668). ^{1}H NMR (CD_3OD, 500 MHz): δ_H 1.60 (1H, m, H-1a), 1.03 (1H, m, H-1b), 1.99 (1H, m, H-2a), 1.74 (1H, m, H-2b), 3.20 (1H, d, $J = 9.5$ Hz, H-3), 0.82 (1H, d, $J = 11.0$ Hz, H-5), 1.66 (1H, m, H-6a), 1.57 (1H, m, H-6b), 1.55 (1H, m, H-7a), 1.42 (1H, m, H-7b), 1.73 (1H, m, H-9), 2.43 (1H, m, H-11a), 2.13 (1H, m, H-11b), 3.32 (1H, d, $J = 9.0$ Hz, H-13), 1.82 (1H, m, H-15a), 1.16 (1H, m, H-15b), 1.92 (1H, m, H-16a), 1.75 (1H, m, H-16b), 2.52 (1H, m, H-17), 1.28 (3H, s, H-18), 1.01 (3H, s, H-19), 1.13 (3H, s, H-21), 1.66 (2H, m, H-22), 2.03 (2H, m, H-23), 5.12 (1H, t, $J = 7.0$ Hz, H-24), 1.70 (3H, s, H-26), 1.64 (3H, s, H-27), 1.10 (3H, s, H-28), 0.90 (3H, s, H-29), 0.76 (3H, s, H-30); *C-3-Glc-1 (inner)*: δ_H 4.44 (1H, d, $J = 7.5$ Hz, H-1′), 3.60 (1H, m, H-2′), 3.33 (1H, m, H-3′), 3.30 (1H, m, H-4′), 3.43 (1H, m, H-5′), 3.84 (1H, br d, $J = 11.0$ Hz, H-6′a), 3.64 (1H, dd, $J = 5.5$, 11.0 Hz, H-6′b); *Glc-2 (term)*: δ_H 4.69 (1H, d, $J = 7.5$ Hz, H-1″), 3.24 (1H, dd, $J = 7.5$, 9.0 Hz, H-2″), 3.36 (1H, m, H-3″), 3.26 (1H, m, H-4″), 3.29 (1H, m, H-5″), 3.86 (1H, dd, $J = 2.5$, 11.0 Hz,

H-6″a), 3.67 (1H, br d, $J = 11.0$ Hz, H-6″b); *C-20-Glc-1 (inner)*: δ_H 4.46 (1H, d, $J = 7.5$ Hz, H-1‴), 3.13 (1H, m, H-2‴), 3.36 (1H, m, H-3‴), 3.37 (1H, m, H-4‴), 3.38 (1H, m, H-5‴), 4.00 (1H, dd, $J = 2.5$, 11.0 Hz, H-6‴a), 3.77 (1H, dd, $J = 5.0$, 11.0 Hz, H6‴b); *Xyl-1 (term)*: δ_H 4.31 (1H, d, $J = 7.5$ Hz, H-1⁗), 3.24 (1H, m, H-2⁗), 3.35 (1H, m, H-3⁗), 3.50 (1H, m, H-4⁗), 3.87 (1H, dd, $J = 2.5$, 11.0 Hz, H-5⁗a), and 3.21 (1H, m, H-5⁗b). ^{13}C NMR (CD_3OD, 125 MHz): δ_C 39.9 (C-1), 27.1 (C-2), 90.9 (C-3), 40.5 (C-4), 57.4 (C-5), 19.3 (C-6), 35.5 (C-7), 41.1 (C-8), 56.3 (C-9), 38.5 (C-10), 40.8 (C-11), 215.5 (C-12), 57.2 (C-13), 57.5 (C-14), 33.0 (C-15), 25.0 (C-16), 43.1 (C-17), 16.3 (C-18), 16.7 (C-19), 82.6 (C-20), 22.7 (C-21), 40.5 (C-22), 24.5 (C-23), 126.0 (C-24), 131.9 (C-25), 25.9 (C-26), 17.8 (C-27), 28.3 (C-28), 16.6 (C-29), 17.1 (C-30); *C-3-Glc-1 (inner)*: δ_C 105.3 (C-1′), 81.2 (C-2′), 77.4 (C-3′), 71.5 (C-4′), 78.5 (C-5′), 63.1 (C-6′); *Glc-2 (term)*: δ_C 104.5 (C-1″), 76.3 (C-2″), 77.8 (C-3″), 71.9 (C-4″), 77.6 (C-5″), 62.8 (C-6″); *C-20-Glc-1 (inner)*: δ_C 98.4 (C-1‴), 75.5 (C-2‴), 78.6 (C-3‴), 71.4 (C-4‴), 76.2 (C-5‴), 70.0 (C-6‴); *Xyl-1 (term)*: δ_C 105.5 (C-1⁗), 74.7 (C-2⁗), 78.3 (C-3⁗), 71.1 (C-4⁗), and 66.7 (C-5⁗).

3.4. Acid hydrolysis of compounds 1–3 and determination of sugar components

The sugar units were identified by acid hydrolysis of compounds followed by GC-MS analysis. This part was conducted according to published protocols with modifications. Details as presented in a previous paper (Thao et al. 2016).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work is supported by the Department of Botany, Hanoi University of Pharmacy. We thank the Institute of Chemistry (VAST) for the NMR experiments.

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References

Chen SK. 1995. A classificatory system and geographical distribution of the genus *Gynostemma* BL. (Cucurbitaceae). Acta Phytotaxon Sin. 33:403–410.

Han J, Gao W, Su D, Liu Y. 2018. Gypenoside inhibits RANKL-induced osteoclastogenesis by regulating NF-κB, AKT, and MAPK signaling pathways. J Cell Biochem. 119:7310–7318. https://doi.org/10.1002/jcb.27028.

Ky PT, Huong PT, My TK, Anh PT, Kiem PV, Minh CV, Cuong NX, Thao NP, Nhiem NX, Hyun JH, Kang KH, Kim YH. 2010. Dammarane-type saponins from *Gynostemma pentaphyllum*. Phytochemistry. 71:994–1001.

Li L, Jiao L, Lau BH. 1993. Protective effect of gypenosides against oxidative stress in phagocytes, vascular endothelial cells and liver microsomes. Cancer Biol Ther. 8:263–272.

Li Y, Lin W, Huang J, Xie Y, Ma W. 2016. Anti-cancer effects of *Gynostemma pentaphyllum* (Thunb.) Makino (Jiaogulan). Chin Med. 11:43.

POWO. 2018. Plants of the World online. [accessed in 2018 Jun 18th]. http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:292896-1.

Razmovski-Naumovski V, Huang HWT, Tran VH, Li GQ, Duke CC, Roufogalis BD. 2005. Chemistry and pharmacology of *Gynostemma pentaphyllum*. Phytochem Rev. 4:197–219.

Sanada S, Shoji J. 1978. Studies on the saponins of ginseng. III. Structures of Ginsenoside-Rb3 and 20-glucoginsenoside-Rf. Chem Pharm Bull. 26:1694–1697.

Seo JY, Kim SK, Nguyen PH, Lee JY, Tung PHT, Sung SH, Oh WK. 2017. Chemical constituents from a *Gynostemma laxum* and their antioxidant and neuroprotective activities. Chin Med. 12:15.

Takemoto T, Arihara S, Nakjima T, Okuhira M. 1983. Studies on the constituents of *Gynostemma pentaphyllum* Makino. I. Structure of gypennoside I-XIV. Yakugaku Zasshi. 103:173–185.

Thao NP, Luyen BTT, Kim JH, Jo AR, Dat NT, Kiem PV, Minh CV, Kim YH. 2016. Identification, characterization, kinetics, and molecular docking of flavonoid constituents from *Archidendron clypearia* (Jack.) Nielsen leaves and twigs. Bioorg Med Chem. 24:3125–3132.

Yahara S, Tanaka O, Komori T. 1976. Saponins of the leaves of *Panax ginseng* C. A. Meyer. Chem Pharm Bull. 24:2204–2208.

Yang H, Kim JY, Kim SO, Yoo YH, Sung. SH. 2014. Complete ^{1}H NMR and ^{13}C NMR spectral analysis of the pairs of 20(*S*) and 20(*R*) ginsenosides. J Ginseng Res. 38:194–202.

Yang TR, Kasai R, Zhou J, Tanaka O. 1983. Dammarane saponins of leaves and seeds of *Panax notoginseng*. Phytochemistry. 22:1473–1478.

Wong WY, Lee MM, Chan BD, Ma VW, Zhang W, Yip TT, Wong WT, Tai WC. 2017. *Gynostemma pentaphyllum* saponins attenuate inflammation *in vitro* and *in vivo* by inhibition of NF-κB and STAT3 signaling. Oncotarget. 8:87401–87414.