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Bidesmoside triterpenoid glycosides from *Stauntonia chinensis* and relationship to anti-inflammation

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ABSTRACT

Ten triterpenoid glycosides, yemuoside YM_{26-35} (**1**–**9** and **12**), were isolated from a traditional Chinese medicine known as "Ye Mu Gua" (*Stauntonia chinensis* DC.) along with two known ones, kalopanax saponin C (**10**) and sieboldianoside A (**11**). Their structures, as elucidated by spectroscopic analyses and chemical methods, were either penta-saccharidic or hexa-saccharidic bidesmoside triterpenoid glycosides. To help explain the clinical applications of "Ye Mu Gua" for its anti-inflammatory effects, the inhibitory activity on the release of inflammatory mediators (nitric oxide, TNF-α and IL-6) of **1**–**12** and the related aglycone, hederagenin (**13**), was evaluated *in vitro*. It was found that compound **13**, but not **1**–**12**, exhibited significant inhibitory activity. The abundant triterpenoid glycosides in "Ye Mu Gua" might therefore be transformed into their respective aglycones, and thus inhibit the release of inflammatory factors *in vivo*. This could then account for the clinical value of "Ye Mu Gua" as regards anti-inflammatory effects. This proposed explanation of how "Ye Mu Gua" may have an effect is similar to the concept of prodrugs for chemical drugs which could be extended to some traditional medicines. That is, the major components might be biologically active not directly, but via biochemical transformation *in vivo*. Hence, we propose a "traditional medicine's prodrug characteristic" concept.

1. Introduction

Stauntonia chinensis DC. has been used as a traditional Chinese medicine, known as "Ye Mu Gua", for its anti-inflammatory and analgesic effects (Jiangsu New Medical College, 1977). This evergreen herb, which grows in southern China and belongs to the Lardizabalaceae, is rich in triterpenoid glycosides (Wang et al., 1989a,b, 1990, 1991). The anti-inflammatory and analgesic effects of preparations of the plant have been evaluated *in vivo* (Peng et al., 2003; Zhang et al., 1998). In our previous studies on the chemical constituents of *S. chinensis*, structures of 10 new saponins were reported (Gao et al., 2007, 2008a,b). Our recent studies on the chemistry and biology of *S. chinensis* led to discovery of many more complex saponins (**1**–**12**). In this paper, the isolation, structural elucidation and relationship to anti-inflammation of **1**–**12** are discussed. The saponins (**1**–**12**) were either penta-saccharidic or hexa-saccharidic bidesmoside triterpenoid glycosides, including 10 new compounds, yemuoside YM_{26-35} (**1**–**9** and **12**). The effects on the inflammation factor-release (nitric oxide, TNF-α and IL-6) of **1**–**12** and the related aglycone, hederagenin (**13**), were evaluated *in vitro*. The structures of **1**–**12** were elucidated by spectroscopic analyses and chemical methods (Fig. 1). Acid hydrolysis of the saponin-enriched fraction yielded the aglycone, hederagenin (**13**), which was identified by comparison with literature NMR spectroscopic data (He et al., 2003). Hederagenin (**13**) exhibited a significant level of inhibitory activity on inflammation factor-release (nitric oxide, TNF-α and IL-6 release), whereas the triterpenoid glycosides **1**–**12** did not. The abundant triterpenoid glycosides of "Ye Mu Gua" might be transformed into their respective aglycones and inhibit inflammation factor-release *in vivo*. This is considered as, at the minimum, one of the reasons to explain the clinical value of "Ye Mu Gua" for its anti-inflammatory effects. It is also one of the ways to clarify the scientific connotation of "Ye Mu Gua". Based on our findings, a "traditional medicine's prodrug characteristic" concept is proposed.

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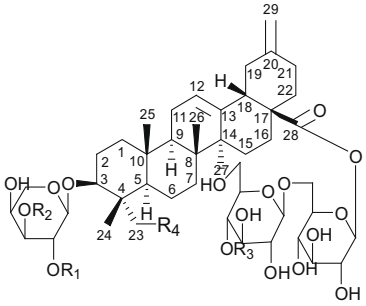


1 R_1=α-L-rhamnopyranosyl
R_2=β-D-xylopyranosyl
R_3=α-L-rhamnopyranosyl
R_4=H

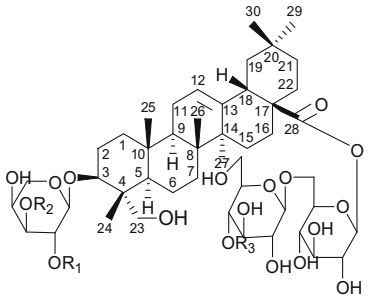
2 R_1=β-D-galactopyranosyl-(1-2)-α-L-rhamnopyranosyl
R_2=H
R_3=α-L-rhamnopyranosyl
R_4=H

3 R_1=α-L-rhamnopyranosyl
R_2=α-L-arabinopyranosyl
R_3=α-L-rhamnopyranosyl
R_4=OH

4 R_1=α-L-rhamnopyranosyl
R_2=β-D-xylopyranosyl
R_3=α-L-rhamnopyranosyl
R_4=OH

5 R_1=α-L-rhamnopyranosyl
R_2=α-L-arabinopyranosyl
R_3=H
R_4=OH

6 R_1=α-L-rhamnopyranosyl
R_2=β-D-xylopyranosyl
R_3=H
R_4=OH



7 R_1=α-L-rhamnopyranosyl
R_2=α-L-arabinopyranosyl
R_3=α-L-rhamnopyranosyl

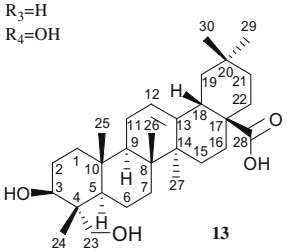
8 R_1=α-L-rhamnopyranosyl
R_2=β-D-xylopyranosyl
R_3=α-L-rhamnopyranosyl

9 R_1=β-D-galactopyranosyl-(1-2)-α-L-rhamnopyranosyl
R_2=H
R_3=α-L-rhamnopyranosyl

10 R_1=α-L-rhamnopyranosyl
R_2=β-D-glucopyranosyl
R_3=α-L-rhamnopyranosyl

11 R_1=β-D-xylopyranosyl-(1-3)-α-L-rhamnopyranosyl
R_2=H
R_3=α-L-rhamnopyranosyl

12 R_1=α-L-rhamnopyranosyl
R_2=α-L-arabinopyranosyl
R_3=H



13

Fig. 1. Structures of **1**–**13**.

2. Results and discussion

Glycosides **1**–**12** were obtained by column chromatography on silica gel, ODS and repeated preparative HPLC in succession from the *n*-BuOH soluble fraction of the aqueous EtOH extract of the rattan of *S. chinensis*. Each compound was obtained as a white amorphous powder, and each gave a positive reaction for the Liebermann-Burchard and Molisch reagents, with each being classified by the type of aglycone: **1**–**2** (akebonic acid), **3**–**6** (30-norhederagenin) and **7**–**12** (hederagenin), respectively. Structural elucidation of the three aglycones was achieved by COSY, HMBC and NOESY spectroscopic analyses of the purified saponins. The ^{13}C NMR spectroscopic data of the three aglycones were in accordance with the corresponding literature (Wang et al., 1989a, 1990, 1991 for akebonic acid; Sbashkov et al., 1998; Yakovishin et al., 1999 for 30-norhederagenin, and Wang et al., 1993a,b for hederagenin).

The molecular formula of glycoside **1** was determined as $C_{63}H_{100}O_{29}$ according to the positive HR-ESI-Q-TOF-MS. After acid hydrolysis and derivatization of **1** as described previously (Hara et al., 1987), GC analysis demonstrated the presence of D-glucose, L-rhamnose, D-xylose and L-arabinose in an approximate ratio of 2:2:1:1, by comparison with derivatives obtained by the same method for standard monosaccharides. The IR spectrum of **1** gave an absorption band at 1743 cm^{-1}, indicating the presence of an ester carbonyl group. In the ESI-IT-MS^n experiments, the MS^2 spectrum of the ion at *m/z* 1343 $[M+Na]^+$ gave positive fragments at *m/z* 873 $[M+Na-470]^+$, 829 $[M+Na-470-44]^+$, 727 $[M+Na-470-146]^+$ and 493 $[470+Na]^+$, whereas that of the ion at *m/z* 1319 $[M-H]^-$ gave negative fragments at *m/z* 849 $[M-H-470]^-$, 717 $[M-H-470-132]^-$, 571 $[M-H-470-132-146]^-$ and 469 $[470-H]^-$. It was suggested that there was a sugar chain connected to **1** by an ester bond, comprising two hexoses (glucoses, considering the results of the GC analysis) and one deoxyhexose (rhamnose, also considering the results of the GC analysis) (470 = 162 + 162 + 146). The reason is that cleavage readily occurs at the ester linkage of glycosides in CID experiments and the charge holds either in the sugar moiety or in the aglycone moiety (Cui et al., 1999). The MS^3 spectrum of the ion at *m/z* 493 $[470+Na]^+$ gave positive fragments at *m/z* 475, 447, 421, 405, 349, 347, 331, 289 and 203, this being in agreement with the sugar linkage pattern of rhamnopyranosyl-(1 → 4)-glucopyranosyl-(1 → 6)-glucopyranosyl (Cui et al., 1999). The MS^3 spectrum of the ion at *m/z* 849 $[M-H-470]^-$ gave negative fragments at *m/z* 717 $[(M-H-470)-132]^-$, 703 $[(M-H-470)-146]^-$, 699 $[(M-H-470)-132\ -18]^-$, 571 $[(M-H-470)-132-146]^-$, 553 $[(M-H-470)$

−132−146−18]$^-$ and 439 [(M−H−470)−132−146−132]$^-$, suggesting presence of another sugar chain ether-linked in **1** comprising two pentoses (one arabinose and one xylose) and one deoxyhexose (rhamnose), considering the results of the GC analysis. In the sugar chain that was ether-linked, the rhamnose and one pentose were at terminal positions, while the other pentose was at an inner position. The ^{1}H and ^{13}C NMR spectroscopic signals of the two sugar chains were assigned based on the analyses of the information obtained from COSY, TOCSY and HSQC experiments. The HMBC correlations found at δ 4.96/δ 69.3 and δ 5.84/δ 78.2 confirmed that the sugar chain ester-linked was rhamnopyranosyl-(1 → 4)-glucopyranosyl-(1 → 6)-glucopyranosyl. The HMBC correlations found at δ 6.10/δ 74.4 and δ 5.02/δ 81.0 indicated that the sugar chain ether-linked was pentopyranosyl-(1 → 3)-[rhamnopyranosyl-(1 → 2)]-pentopyranosyl. For the terminal pentose unit in the sugar chain that was ether-linked, comparison of the ^{13}C NMR spectroscopic data to those of various monosaccharides suggested that the terminal pentose was β-xylopyranosyl (Bock and Pedersen, 1983). Thus, the sugar chain ether-linked was considered to be xylopyranosyl-(1 → 3)-[rhamnopyranosyl-(1 → 2)]-arabinopyranosyl. According to anomeric proton signals, the relative configurations of the sugar units were determined as β-glucose, α-rhamnose, β-xylose and α-arabinose. The HMBC correlation found at δ 6.19/δ 175.7 and the NOESY correlation found at δ 4.93/δ 3.30 indicated that the sugar chain ester-linked was attached to C-28 of the aglycone and that the sugar chain ether-linked was attached to C-3. Hence, **1** was determined to be 3-*O*-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-akebonic acid-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{26}$.

The molecular formulae of glycosides **4** and **8** were determined as $C_{63}H_{100}O_{30}$ and $C_{64}H_{104}O_{30}$ according to the positive HR-ESI-Q-TOF-MS, respectively. After acid hydrolysis and derivatization of **4** and **8**, GC analysis gave the same results as for **1**. Comparison of the ^{13}C NMR spectroscopic data of **4** and **8**, to those of the moieties of two sugar chains of **1**, suggested that **4** and **8** possessed the same two sugar chains as in **1**. This deduction was confirmed by the ESI-IT-MS^n experiments, as well as by IR and 2D-NMR spectroscopic analyses. The HMBC correlation found at δ 6.18/δ 175.7 and the NOESY correlation observed at δ 5.14/δ 4.31 in **4**, and the HMBC correlation noted at δ 6.22/δ 176.5 and the NOESY correlation at δ 5.13/δ 4.29 in **8**, indicated that the sugar chain ester-linked was attached at C-28 of the aglycone and that the sugar chain ether-linked was attached at C-3 in **4** and **8**. Hence, **4** was determined to be 3-*O*-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{29}$, and **8** to be 3-*O*-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{33}$.

By the positive HR-ESI-Q-TOF-MS, the molecular formula of glycoside **6** was determined as $C_{57}H_{90}O_{26}$. After acid hydrolysis and derivatization of **6**, GC analysis gave D-glucose, L-rhamnose, D-xylose and L-arabinose in an approximate ratio of 2:1:1:1. In the MS^2 spectra, the ion at *m*/*z* 1213 [M+Na]$^+$ gave positive fragments at *m*/*z* 889 [M+Na−324]$^+$ and 743 [M+Na−324−146]$^+$, and the ion at *m*/*z* 1189 [M−H]$^-$ gave negative fragments at *m*/*z* 865 [M−H−324]$^-$ and 733 [M−H−324−132]$^-$. The loss of 324 (162 + 162) mass units indicated the presence of a sugar chain ester-linked in **6**, comprising two glucoses moieties, considering the results of the GC analysis. The absorption band at 1743 cm^{-1} in the IR spectrum of **6** also indicated the presence of an ester carbonyl group. The assignment of ^{1}H and ^{13}C NMR spectroscopic signals of the sugar chain ester-linked was achieved by analysing of the COSY, TOCSY and HSQC correlations. The HMBC cross-peak observed at δ 5.00/δ 69.5 indicated that the sugar chain ester-linked was glucopyranosyl-(1 → 6)-glucopyranosyl. The remaining ^{13}C NMR spectroscopic signals of **6** were the same as those except the moiety of the sugar chain ester-linked of **4**, suggesting that **6** possessed the same aglycone and the same sugar chain ether-linked as in **4**. This deduction was confirmed by the ESI-IT-MS^n experiments and 2D-NMR spectroscopic analyses. The relative configurations of the sugar units were determined as β-glucose, α-rhamnose, β-xylose and α-arabinose by anomeric proton signals. The HMBC correlation observed at δ 6.20/δ 175.7 and the NOESY correlation found at δ 5.13/δ 4.30 indicated that the sugar chain ester-linked was attached at C-28 of the aglycone and that the sugar chain ether-linked was attached at C-3. Hence, **6** was determined to be 3-*O*-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{31}$.

The molecular formula of glycoside **3** could be determined as $C_{63}H_{100}O_{30}$ by the positive HR-ESI-Q-TOF-MS. The acid hydrolysis, derivatization and GC analysis suggested the presence of D-glucose, L-rhamnose and L-arabinose in an approximate ratio of 1:1:1 in **3**. The MS^2 spectrum of the ion at *m*/*z* 1359 [M+Na]$^+$ gave positive fragments at *m*/*z* 889 [M+Na−470]$^+$, 743 [M+Na−470−146]$^+$ and 493 [470+Na]$^+$, whereas that of the ion at *m*/*z* 1335 [M−H]$^-$ gave negative fragments at *m*/*z* 865 [M−H−470]$^-$, 733 [M−H−470−132]$^-$ and 719 [M−H−470−146]$^-$. The loss of 470 mass units suggested the presence of a sugar chain ester-linked in **3**, comprising two glucoses and one rhamnose, considering the results of the GC analysis, as for **1**. The presence of an ester carbonyl group could also be deduced by the absorption band at 1743 cm^{-1} in the IR spectrum of **3**. The fragment pattern of the MS^3 spectrum of the ion at *m*/*z* 493 [470+Na]$^+$ was the same as in **1**. Comparison of the ^{13}C NMR spectroscopic data of **3** to those of the moiety of the sugar chain ester-linked of **1** suggested that **3** possessed the same sugar chain ester-linked as in **1**. This deduction was confirmed by the 2D-NMR spectroscopic analyses. The MS^3 spectrum of the ion at *m*/*z* 865 [M−H−470]$^-$ gave negative fragments at *m*/*z* 733 [(M−H−470)−132]$^-$, 719 [(M−H−470)−146]$^-$, 587 [(M−H−470)−132−146]$^-$, 569 [(M−H−470)−132−146−18]$^-$ and 455 [(M−H−470)−132−146−132]$^-$, suggesting presence of another sugar chain ether-linked in **3** comprising two arabinoses and one rhamnose, considering the results of the GC analysis. In the sugar chain ether-linked, the rhamnose and one arabinose were at the terminal positions, while the other arabinose was at the inner position. Based on COSY, TOCSY and HSQC correlations, the assignment of ^{1}H and ^{13}C NMR signals of the sugar chain ester-linked was achieved. The correlation at δ 6.09/δ 74.4 in HMBC and the correlation at δ 4.97/δ 4.13 in NOESY indicated that the sugar chain ether-linked was arabinopyranosyl-(1 → 3)-[rhamnopyranosyl-(1 → 2)]-arabinopyranosyl. The relative configurations of β-glucose, α-rhamnose and α-arabinose could be determined by analysis of anomeric proton signals. The correlation at δ 6.19/δ 175.7 in the HMBC, and the correlation at δ 5.16/δ 4.28 in the NOESY spectrum indicated that the sugar chain ester-linked was attached at C-28 of the aglycone and that the sugar chain ether-linked was attached at C-3. Hence, **3** was determined to be 3-*O*-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{28}$.

For glycoside **7**, the molecular formula was $C_{64}H_{104}O_{30}$ judged from the positive HR-ESI-Q-TOF-MS. After acid hydrolysis and derivatization of **7**, GC analysis gave the same results as for **3**. Comparison of the ^{13}C NMR spectroscopic data of **7** to those of the moieties of two sugar chains of **3** suggested that **7** possessed the same

two sugar chains as in **3**. This was confirmed by the ESI-IT-MS^n experiments, IR and 2D-NMR spectroscopic analyses. The cross-peak at δ 6.23/δ 176.4 in HMBC and the NOESY correlation found at δ 5.15/δ 4.27 indicated that the sugar chain ester-linked was attached at C-28 of the aglycone, and that the sugar chain ether-linked was attached at C-3. Hence, **7** was determined to be 3-*O*-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{32}$.

From the positive HR-ESI-Q-TOF-MS, the molecular formula of glycoside **5** was determined as $C_{57}H_{90}O_{26}$. The acid hydrolysis, derivatization and GC analysis gave D-glucose, L-rhamnose and L-arabinose in an approximate ratio of 2:1:2 in **5**. Its IR spectrum gave an absorption band at 1739 cm^{-1} for an ester carbonyl group. In the MS^2 spectra, the ion at *m*/*z* 1213 [M+Na]$^+$ gave positive fragments at *m*/*z* 889 [M+Na−324]$^+$ and 743 [M+Na−324−146]$^+$, whereas that of the ion at *m*/*z* 1189 [M−H]$^-$ gave negative fragments at *m*/*z* 865 [M−H−324]$^-$, 733 [M−H−324−132]$^-$ and 719 [M−H−324−146]$^-$. The loss of 324 mass units suggested the presence of a sugar chain ester-linked in **5**, comprising two glucoses as in **6**. Comparison of the ^{13}C NMR spectroscopic data of **5** to those of the moiety of the sugar chain ester-linked of **6** suggested that **5** possessed the same sugar chain ester-linked as in **6**. The remaining ^{13}C NMR spectroscopic signals of **5** were the same as those except the moiety of the sugar chain ester-linked of **3**, suggesting that **5** possessed the same aglycone and the same sugar chain ether-linked as in **3**. The results from ESI-IT-MS^n experiments and 2D-NMR spectroscopic analyses supported the above reasoning. The sugar chain ester-linked was attached at C-28 of the aglycone and the sugar chain ether-linked was attached at C-3, as judged from the HMBC correlation at δ 6.20/δ 175.7 and the NOESY correlation at δ 5.15/δ 4.28, respectively. Hence, **5** was determined to be 3-*O*-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-30-norhederagenin-28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{30}$.

The positive HR-ESI-Q-TOF-MS gave a molecular formula of $C_{58}H_{94}O_{26}$ for glycoside **12**. Acid hydrolysis, derivatization and GC analysis of **12** gave the same results as for **5**. By comparison of the ^{13}C NMR spectroscopic data of **12** to those of the moieties of two sugar chains of **5**, **12** possessed the same two sugar chains as in **5**, which was supported by the ESI-IT-MS^n experiments, IR and 2D-NMR spectroscopic analyses. The linkage of the sugar chain ester-linked at C-28 of the aglycone was deduced by the correlation at δ 6.25/δ 176.4 in HMBC, whereas the linkage of the sugar chain ether-linked at C-3 was indicated by the correlation at δ 5.15/δ 4.28 in NOESY. Hence **12** was determined to be 3-*O*-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-hederagenin-28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{35}$.

The molecular formula of glycoside **2** was determined as $C_{64}H_{102}O_{30}$ according to the positive HR-ESI-Q-TOF-MS. After acid hydrolysis and derivatization of **2**, GC analysis demonstrated the presence of D-glucose, D-galactose, L-rhamnose and L-arabinose in an approximate ratio of 2:1:2:1. There was an absorption band at 1743 cm^{-1} in the IR spectrum of **2** for an ester carbonyl group. In the MS^2 spectra, the loss of 470 mass units suggested the presence of a sugar chain ester-linked in **2**, comprising two hexoses and one deoxyhexose. Comparison of the ^{13}C NMR spectroscopic data of **2** to those of the moiety of the sugar chain ester-linked of **1** suggested that **2** possessed the same sugar chain ester-linked as in **1**. The MS^3 spectrum of the ion at *m*/*z* 879 [M−H−470]$^-$ gave negative fragments at *m*/*z* 835 [(M−H−470)−44]$^-$, 717 [(M−H−470)−162]$^-$, 699 [(M−H−470)−162−18]$^-$, 571 [(M−H−470)−162−146]$^-$, 553 [(M−H−470)−162−146−18]$^-$ and 439 [(M−H−470)−162−146−132]$^-$, suggesting presence of another sugar chain ether-linked in **2** comprising one arabinose, one rhamnose and one galactose, considering the results of the GC analysis and the deduced structure of the sugar chain ester-linked. In the sugar chain ether-linked, the galactose was at the terminal position, while the arabinose was at the inner position. The assignment of the ^{1}H and ^{13}C NMR spectroscopic signals of the two sugar chains could be completed by the analyses of the information of COSY, TOCSY and HSQC experiments. The cross-peaks at δ 6.34/δ 76.8 and δ 5.16/δ 82.5 in HMBC indicated that the sugar chain ether-linked was galactopyranosyl-(1 → 2)-rhamnopyranosyl-(1 → 2)-arabinopyranosyl. The relative configurations of β-glucose, β-galactose, α-rhamnose and α-arabinose were deduced by anomeric proton signals. The HMBC correlation observed at δ 6.18/δ 175.8 and the NOESY correlation found at δ 4.80/δ 3.25 indicated that the sugar chain ester-linked was attached at C-28 of the aglycone and that the sugar chain ether-linked was attached at C-3. Hence, **2** was determined to be 3-*O*-β-D-galactopyranosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-akebonic acid-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{27}$.

For glycoside **9**, the molecular formula of $C_{65}H_{106}O_{31}$ was deduced according to the positive HR-ESI-Q-TOF-MS. Acid hydrolysis, derivatization and GC analysis of **9** gave the same results as for **2**. Compound **9** had the same two sugar chains as in **2** by comparison of the ^{13}C NMR spectroscopic data of **9** to those of the moieties of two sugar chains of **2**. The HMBC cross-peak at δ 6.23/δ 176.4 and the NOESY correlation at δ 5.03/δ 4.25 indicated that the sugar chain ester-linked was attached at C-28 of the aglycone, and that the sugar chain ether-linked was attached to C-3. Hence, **9** was determined to be 3-*O*-β-D-galactopyranosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, named yemuoside YM$_{34}$.

The structures of the known glycosides (**10** and **11**) were elucidated by spectroscopic analyses and identified by comparisons of their physical and spectral properties with references to be 3-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (kalopanax saponin C) (**10**) (Shao et al., 1989) and 3-*O*-β-D-xylopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (sieboldianoside A) (**11**) (Panov et al., 2006).

To explain the anti-inflammatory effects of "Ye Mu Gua", the *in vitro* effects of the glycosides **1**–**12** on the inflammatory response were investigated. The anti-inflammatory effects were evaluated by the inhibitory activity on lipopolysaccharide-stimulated (LPS-stimulated) inflammation factor-release (nitric oxide-release, TNF-α-release and IL-6-release) of mouse monocyte-macrophages RAW 264.7 *in vitro*. Nitric oxide-release was determined by measuring the amount of nitrite in the cell culture supernatant using Griess reagent (Lee et al., 1999) and, at the same time, cell viability was evaluated using the MTT method (Denizot and Lang, 1986). TNF-α- and IL-6-releases were detected in the cell culture supernatant using the respective enzyme-linked immunosorbent assay kits (mouse TNF-α ELISA kit and mouse IL-6 ELISA kit), according to the respective manufacturer's instructions. For all assays, hydrocortisone was used as a positive control. The IC$_{50}$ values of hydrocortisone for the assays were measured as 64.3 μM for the inhibitory activity on nitric oxide-release, 85.6 μM for the inhibitory activity on TNF-α-release and 63.8 μM for the inhibitory activity on IL-6-release, respectively. However, compounds **1**–**12** did not exhibit inhibitory activity on inflammation factor-release *in vitro*. Thus, we evaluated hederagenin (**13**) from the saponin-enriched fraction by acid hydrolysis on the inhibitory activity. *In vitro*,

hederagenin (**13**) exhibited a significant inhibitory level of inhibitory activity on the inflammation factor-release. The IC_{50} values of hederagenin (**13**) were measured as 8.29 μM for the inhibitory activity on nitric oxide-release, 7.32 μM for the inhibitory activity on TNF-α-release, and 8.59 μM for the inhibitory activity on IL-6-release, respectively. The IC_{50} value of hederagenin (**13**) for the inhibitory activity on nitric oxide-release was, however, less than the results reported by Kim et al. (2002). As a plant of the Lardizabalacea, "Ye Mu Gua" is rich in triterpenoid glycosides, but it is not clear how these compounds cause the anti-inflammatory effects in this medicine. Based on the results of the inhibitory activity of these inflammation factors release *in vitro*, the triterpenoid glycosides of "Ye Mu Gua" might be transformed into their respective aglycones and thereby inhibit inflammation factor-release *in vivo*. This is considered as, at the minimum, one of the reasons to explain the clinical value of "Ye Mu Gua" for its anti-inflammatory effects.

2.1. Concluding remarks

This consideration of how "Ye Mu Gua" has an effect is similar to the concept of prodrug for chemical drugs. In general, chemical diversity, functional diversity and synergism are considered to be characteristics of traditional medicines. However, in addition, some feature similar to a prodrug may be of importance for some traditional medicines, such as "Ye Mu Gua". For such traditional medicines, the major components might be biologically active not directly, but by biochemical transformation to a few chemical entities *in vivo*. This would be similar to the concept of a prodrug and which we thus describe as a "traditional medicine's prodrug characteristics". Taking "Ye Mu Gua" as an example, the major components are saponins which are highly diverse in structure. The transformation from saponins to a few aglycones decreases the chemical diversity, but provides a means to take effect in the traditional medicine. Thus, for some traditional medicines, the prodrug characteristic is usually important for understanding, to some extent, the way of how the effect occurs.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured using a JASCO P-1020 polarimeter, and IR and NMR spectra were acquired using a JASCO FT/IR-480 plus spectrometer, and a Bruker AVANCE 400 NMR spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C), respectively. ESI-IT-MS^n spectra were obtained using a Bruker esquire 2000 mass spectrometer, and HR-ESI-Q-TOP-MS were acquired with a Micromass Q-TOF mass spectrometer. Analytical HPLC was performed using a Shim-pack VP-ODS column (4.6 × 250 mm), whereas preparative HPLC was employed on a Shim-pack PRC-ODS column (20 × 250 mm) with a refractive index detector (RID-10A). Column chromatographic (CC) purifications were carried out on silica gel (200–300 mesh) (Qingdao Haiyang Chemical Group Corporation, China), Sephadex LH-20 (Amersham Biosciences) and ODS (60–80 μm, Merck), respectively. Silica gel GF_{254} (Qingdao Haiyang Chemical Group Corporation, China) and RP-18 F_{254} (Merck) were used for analytical TLC. GC analysis was carried out using a Varian CP-3800 GC with a HP-1701 column (0.25 mm × 30 m).

3.2. Plant material

The rattan of *S. chinensis* DC. was collected in Jiangxi Province by LifeTech Pharmaceuticals Ltd. (Guangzhou) in November 2004, and identified by the Traditional Chinese Medicine Department at the Shenzhen Institute of Drug Control. A voucher specimen (YMG-2004-JX-DN) is deposited at Research Center of Traditional Chinese Medicine and Natural Products, Shenzhen, China.

3.3. Extraction and isolation

The air-dried rattan of *S. chinensis* (20 kg) was chopped into small pieces, then extracted with EtOH–H_2O (60:40, v/v) (200 L) by heating the suspension until reflux began, this being maintained for 2 h. This procedure was next repeated. After evaporation of the combined EtOH extracts *in vacuo*, the resultant aqueous residue was extracted with *n*-BuOH (20 L × 3). A portion (150 g dry extract) of the combined *n*-BuOH extract, following solvent removal, was subjected to silica gel CC, eluted with a $CHCl_3$–MeOH–H_2O gradient. The step gradient sequence was 100:0:0, 98:2:0, 95:5:0, 90:10:0, 80:20:2, 70:30:5, 60:40:8, and 0:100:0 (v/v), respectively, with five column volumes for each solvent step applied, with the total volume of eluent from each step was collected as one fraction. The saponin-enriched fraction ($CHCl_3$–MeOH–H_2O 60:40:8, v/v) was subjected to ODS CC, eluted with a MeOH–H_2O gradient. The step gradient sequence was 0:100, 10:90, 30:70, 50:50, 70:30, 90:10, and 100:0 (v/v), with five column volumes for each elution step; the total volume of eluent from each step collected as one subfraction. Subfraction 5 (MeOH–H_2O 70:30, v/v) was isolated by preparative HPLC with MeOH–H_2O (65:35, v/v) using a refractive index detector for detection to yield 14 fine subfractions by retention-time order. Fine subfraction 3 was purified further isolated by preparative HPLC with MeOH–H_2O (60:40, v/v) as eluent to yield **3** (9.5 mg) and **4** (14.3 mg), whereas fine subfraction 4 yielded **5** (10.6 mg), **6** (10.0 mg) and **10** (8.2 mg) using the identical chromatographic conditions. In an analogous manner, fine subfraction 5 afforded **9** (10.0 mg) and **11** (19.6 mg), fine subfraction 6 gave **7** (16.5 mg) and **8** (29.4 mg), and fine subfraction 7 afforded **12** (4.2 mg). Fine subfraction 10 was subjected to preparative HPLC with MeOH–H_2O (65:35, v/v) as eluent to yield **2** (95.1 mg), whereas fine subfraction 11, treated in the same way, yielded **1** (11.4 mg). In all cases, the refractive index detector was employed for detection.

3.3.1. 3-O-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-akebonic acid-28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**1**)

White amorphous powder; $[\alpha]_D^{27}$ 3.1 (c 0.11, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3452, 2938, 1743, 1650, 1458, 1390, 1042; for ^1H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 1; +ESI-IT-MS *m/z*: 1343 $[M+Na]^+$; +ESI-IT-MS^2 (1343) *m/z*: 873 $[M+Na-470]^+$, 829 $[M+Na-470-44]^+$, 727 $[M+Na-470-146]^+$, 493 $[470+Na]^+$; +ESI-IT-MS^3 (1343-873) *m/z*: 829 $[(M+Na-470)-44]^+$, 741 $[(M+Na-470)-132]^+$, 727 $[(M+Na-470)-146]^+$, 697 $[(M+Na-470)-132-44]^+$, 683 $[(M+Na-470)-146-44]^+$; +ESI-IT-MS^3 (1343-493) *m/z*: 475, 447, 421, 405, 349, 347, 331, 289, 203; -ESI-IT-MS *m/z*: 1319 $[M-H]^-$; -ESI-IT-MS^2 (1319) *m/z*: 849 $[M-H-470]^-$, 717 $[M-H-470-132]^-$, 571 $[M-H-470-132-146]^-$, 469 $[470-H]^-$; -ESI-IT-MS^3 (1319-849) *m/z*: 717 $[(M-H-470)-132]^-$, 703 $[(M-H-470)-146]^-$, 699 $[(M-H-470)-132-18]^-$, 571 $[(M-H-470)-132-146]^-$, 553 $[(M-H-470)-132-146-18]^-$, 439 $[(M-H-470)-132-146-132]^-$; -ESI-IT-$MS^3$ (1319−717) *m/z*: 571 $[(M-H-470-132)-146]^-$, 553 $[(M-H-470-132)-146-18]^-$, 439 $[(M-H-470-132)-146-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1343.6199 $[M+Na]^+$ (calcd. for $C_{63}H_{100}NaO_{29}$, 1343.6248).

3.3.2. 3-O-β-D-galactopyranosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-akebonic acid-28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**2**)

White amorphous powder; $[\alpha]_D^{27}$ 7.9 (c 0.17, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3453, 2939, 1743, 1656, 1456, 1386, 1073; for ^1H

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data in pyridine-d_5 for **1**–**5**.

No.	**1**		**2**		**3**		**4**		**5**	
	δ_C	δ_H (*J*)	δ_C	δ_H (*J*)	δ_C	δ_H (*J*)	δ_C	δ_H (*J*)	δ_C	δ_H (*J*)
1	39.0	0.93; 1.48	38.9	0.91; 1.47	39.1	1.03; 1.52	39.1	1.05; 1.54	39.1	1.04; 1.52
2	26.5	1.83; 2.04	26.5	1.81; 2.05	26.1	1.96; 2.14	26.2	2.01; 2.18	26.1	1.96; 2.15
3	88.2	3.30 (*dd*, 11.7, 3.7)	88.6	3.25 (*dd*, 11.2, 3.5)	80.5	4.28	80.6	4.31	80.5	4.28
4	39.5		39.4		43.4		43.4		43.4	
5	55.9	0.76 (*d*, 11.0)	55.9	0.76 (*d*, 11.0)	47.6	1.69	47.7	1.71	47.6	1.70
6	18.5	1.30; 1.44	18.5	1.31; 1.44	18.0	1.36	18.0	1.38	18.0	1.41
7	33.0	1.30; 1.44	33.0	1.30; 1.44	32.7	1.27; 1.56	32.7	1.28; 1.58	32.7	1.28; 1.57
8	39.8		39.9		39.8		39.9		39.9	
9	48.0	1.59	48.0	1.58	48.1	1.69	48.1	1.71	48.1	1.70
10	37.0		36.9		36.8		36.8		36.8	
11	23.7	1.84	23.7	1.84	23.7	1.86	23.7	1.86	23.7	1.87
12	123.3	5.42 (*t*, 3.6)	123.3	5.42 (*t*, 3.6)	123.3	5.41 (*t*, 3.4)	123.3	5.41 (*t*, 3.6)	123.3	5.40 (*t*, 3.7)
13	143.4		143.4		143.3		143.3		143.3	
14	42.0		42.1		42.0		42.0		42.0	
15	28.2	1.16; 2.30 (*br. t*, 12.5)	28.2	1.15; 2.28 (*br. t*, 11.4)	28.2	1.08; 2.26 (*td*, 13.6, 3.3)	28.2	1.09; 2.27 (*td*, 13.5, 3.3)	28.2	1.10; 2.28 (*td*, 13.3, 3.3)
16	23.5	2.03; 2.14	23.5	2.03; 2.15	23.4	1.98; 2.08	23.4	1.98; 2.08	23.4	2.00; 2.08
17	47.3		47.3		47.3		47.3		47.3	
18	47.5	3.10 (*dd*, 13.3, 4.1)	47.5	3.09 (*dd*, 13.7, 4.6)	47.4	3.08 (*dd*, 13.3, 4.4)	47.4	3.08 (*dd*, 13.1, 4.3)	47.4	3.09 (*dd*, 13.3, 4.3)
19	41.6	2.17; 2.56 (*t*, 13.3)	41.7	2.16; 2.56 (*t*, 13.4)	41.6	2.13; 2.51 (*t*, 13.3)	41.6	2.14; 2.51 (*t*, 13.3)	41.6	2.13; 2.52 (*t*, 13.6)
20	148.3		148.3		148.3		148.3		148.3	
21	30.0	2.08; 2.17	30.1	2.06; 2.18	30.0	2.05; 2.14	30.0	2.06; 2.15	30.0	2.05; 2.15
22	37.6	1.72; 2.02	37.6	1.71; 2.02	37.6	1.71; 2.00	37.6	1.71; 1.99	37.6	1.73; 2.02
23	28.1	1.18 (*s*)	28.0	1.17 (*s*)	63.9	3.73; 4.11	63.8	3.75; 4.14	63.9	3.74; 4.11
24	17.0	1.11 (*s*)	16.9	1.05 (*s*)	13.8	1.06 (*s*)	13.9	1.09 (*s*)	13.8	1.05 (*s*)
25	15.6	0.86 (*s*)	15.6	0.86 (*s*)	16.2	0.94 (*s*)	16.2	0.95 (*s*)	16.2	0.94 (*s*)
26	17.4	1.06 (*s*)	17.5	1.06 (*s*)	17.5	1.08 (*s*)	17.5	1.09 (*s*)	17.5	1.09 (*s*)
27	26.0	1.22 (*s*)	26.0	1.21 (*s*)	25.9	1.12 (*s*)	25.9	1.13 (*s*)	25.9	1.13 (*s*)
28	175.7		175.8		175.7		175.7		175.7	
29	107.3	4.67; 4.74	107.3	4.68 (*s*); 4.74 (*s*)	107.3	4.66; 4.72 (*s*)	107.3	4.66; 4.72	107.2	4.63; 4.69
28 Sugar chain										
	28-*O*-*Glc*		28-*O*-*Glc*		28-*O*-*Glc*		28-*O*-*Glc*		28-*O*-*Glc*	
1	95.7	6.19 (*d*, 8.1)	95.7	6.18 (*d*, 7.9)	95.7	6.19 (*d*, 8.1)	95.7	6.18 (*d*, 8.3)	95.7	6.20 (*d*, 8.0)
2	73.8	4.10	73.8	4.10	73.9[a]	4.09	73.8	4.09	73.8[a]	4.09
3	78.6	4.18	78.6	4.18	78.6	4.18	78.6	4.19	78.6	4.19

4	70.8	4.26	70.8	4.25	70.8	4.26	70.8	4.26	70.9	4.29
5	77.9	4.07	77.9	4.05	77.9	4.06	77.9	4.08	77.8	4.07
6	69.3	4.30; 4.64	69.3	4.29; 4.63	69.3	4.29; 4.64	69.3	4.29; 4.66	69.5	4.32; 4.68
	28-*O*-Glc-6-*Glc*		28-*O*-Glc-6-*Glc*		28-*O*-Glc-6-*Glc*		28-*O*-Glc-6-*Glc*		28-*O*-Glc-6-*Glc*	
1	104.9	4.96 (*d*, 8.2)	104.9	4.95 (*d*, 7.2)	104.9	4.96 (*d*, 7.7)	104.9	4.96 (*d*, 8.1)	105.3	5.00 (*d*, 7.8)
2	75.3	3.93 (*t*, 8.1)	75.3	3.93 (*t*, 8.5)	75.2	3.93 (*t*, 8.4)	75.3	3.93 (*t*, 8.2)	75.1	4.00 (*t*, 7.9)
3	76.5	4.14	76.5	4.13	76.4	4.14	76.4	4.15	78.4[b]	4.19
4	78.2	4.41 (*t*, 9.3)	78.2	4.38	78.2	4.40	78.2	4.40	71.5	4.20
5	77.1	3.66	77.1	3.65 (*br. d*, 9.5)	77.1	3.66	77.1	3.66	78.3[b]	3.88
6	61.3	4.09; 4.20	61.3	4.08; 4.20	61.3	4.08; 4.20	61.3	4.09; 4.21	62.6	4.35; 4.47
	28-*O*-Glc-6-Glc-4-*Rha*		28-*O*-Glc-6-Glc-4-*Rha*		28-*O*-Glc-6-Glc-4-*Rha*		28-*O*-Glc-6-Glc-4-*Rha*			
1	102.7	5.84 (*br. s*)	102.7	5.83 (*br. s*)	102.7	5.84 (*br. s*)	102.6	5.84 (*br. s*)		
2	72.5	4.67	72.5	4.66	72.5	4.67	72.5	4.67		
3	72.7	4.56	72.7	4.53	72.7	4.56	72.7	4.54 (*dd*, 9.4, 2.9)		
4	73.9	4.29	73.9	4.31	73.9	4.31	73.9	4.32		
5	70.2	4.95	70.3	4.94	70.2	4.95	70.2	4.95		
6	18.5	1.69 (*d*, 6.1)	18.5	1.68 (*d*, 5.9)	18.5	1.69 (*d*, 6.2)	18.4	1.69 (*d*, 6.0)		
3 Sugar chain										
	3-*O*-*Ara*		3-*O*-*Ara*		3-*O*-*Ara*		3-*O*-*Ara*		3-*O*-*Ara*	
1	104.5	4.93 (*d*, 5.2)	104.9	4.80 (*d*, 5.5)	103.9	5.16 (*d*, 5.8)	104.2	5.14 (*d*, 6.2)	103.9	5.15 (*d*, 5.9)
2	74.4[a]	4.66	76.8	4.43	74.4	4.64	74.3	4.67	74.5	4.63
3	81.0	4.32	74.4	4.15	81.1	4.13	82.5	4.07	81.2	4.13
4	68.0	4.48	69.0	4.20	68.3	4.40	68.8	4.38	68.4	4.39
5	64.5	3.81 (*br. d*, 12.1); 4.31	65.4	3.77 (*br. d*, 10.7); 4.26	65.1	3.69; 4.25	65.5	3.71; 4.26	65.2	3.69; 4.25
	3-*O*-Ara-2-*Rha*		3-*O*-Ara-2-*Rha*		3-*O*-Ara-2-*Rha*		3-*O*-Ara-2-*Rha*		3-*O*-Ara-2-*Rha*	
1	102.0	6.10 (*br. s*)	100.9	6.34 (*br. s*)	101.9	6.09 (*br. s*)	101.8	6.19 (*br. s*)	101.9	6.08 (*br. s*)
2	72.4	4.71	82.5	4.77	72.3	4.69	72.3	4.71	72.3	4.69
3	72.5	4.56	72.5	4.58	72.6	4.56	72.5	4.58 (*dd*, 9.4, 2.9)	72.6	4.57 (*dd*, 9.2, 3.1)
4	73.9	4.29	74.4	4.12	74.0	4.25	73.9	4.25	73.9[a]	4.24
5	70.0	4.57	69.5	4.53	70.0	4.64	69.9	4.68	70.0	4.63
6	18.5	1.63 (*d*, 6.1)	18.5	1.56 (*d*, 5.4)	18.4	1.65 (*d*, 6.2)	18.5	1.64 (*d*, 6.1)	18.5	1.64 (*d*, 6.1)
	3-*O*-Ara-3-*Xyl*		3-*O*-Ara-2-Rha-2-*Gal*		3-*O*-Ara-3-*Ara*		3-*O*-Ara-3-*Xyl*		3-*O*-Ara-3-*Ara*	
1	104.9	5.02 (*d*, 7.2)	107.9	5.16 (*d*, 7.6)	104.7	4.97 (*d*, 6.3)	105.5	4.93 (*d*, 7.4)	104.8	4.97 (*d*, 6.5)
2	74.5[a]	3.93 (*t*, 8.1)	73.3	4.50	72.2	4.46 (*t*, 7.7)	74.5	3.93 (*t*, 8.2)	72.2	4.46
3	77.6	4.07	75.3	4.11	73.8[a]	4.12	77.8	4.08	74.0	4.13
4	70.8	4.10	70.3	4.45	68.8	4.33	70.8	4.10	68.8	4.33
5	66.9	3.65; 4.30	77.2	3.99 (*t*, 5.9)	66.4	3.74; 4.32	67.0	3.64; 4.29	66.4	3.76; 4.32
6			62.3	4.28; 4.36						

[a,b]These assignments with the same superscript may be interchanged in each column.

Table 2
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data in pyridine-d_5 for **6**–**9** and **12**.

No.	**6**		**7**		**8**		**9**		**12**	
	δ_C	δ_H (*J*)	δ_C	δ_H (*J*)	δ_C	δ_H (*J*)	δ_C	δ_H (*J*)	δ_C	δ_H (*J*)
1	39.1	1.05; 1.53	39.0	1.04; 1.54	39.1	1.05; 1.54	39.0	1.04; 1.54	39.0	1.04; 1.54
2	26.2	2.00; 2.18	26.2	1.97; 2.15	26.2	2.00; 2.17	26.2	1.98; 2.18	26.2	1.96; 2.14
3	80.6	4.30	80.5	4.27	80.6	4.29	80.9	4.25	80.5	4.28
4	43.4		43.4		43.5		43.4		43.4	
5	47.7	1.71	47.7	1.72	47.7	1.72	47.6	1.72	47.6	1.72
6	18.0	1.41	18.1	1.37	18.1	1.37	18.1	1.37	18.1	1.37
7	32.7	1.28; 1.58	32.7	1.29; 1.57	32.7	1.29; 1.58	32.7	1.30; 1.58	32.7	1.29; 1.57
8	39.9		39.8		39.9		39.8		39.8	
9	48.1	1.71	48.1	1.72	48.2	1.72	48.1	1.72	48.1	1.72
10	36.8		36.8		36.9		36.8		36.8	
11	23.7	1.88	23.8	1.90	23.8	1.90	23.8	1.90	23.7	1.91
12	123.3	5.40 (*t*, 3.6)	122.9	5.39 (*t*, 3.7)	122.9	5.39 (*t*, 3.7)	122.9	5.38 (*t*, 3.7)	122.9	5.40 (*t*, 3.7)
13	143.3		144.0		144.0		144.0		144.0	
14	42.0		42.1		42.1		42.1		42.1	
15	28.2	1.09; 2.28 (*br. t*, 13.3)	28.2	1.06; 2.26 (*br. t*, 13.3)	28.3	1.06; 2.26 (*br. t*, 12.9)	28.2	1.06; 2.26 (*br. t*, 13.3)	28.2	1.06; 2.28 (*br. t*, 13.6)
16	23.4	2.00; 2.07	23.3	1.90; 1.99	23.3	1.90; 2.00	23.3	1.89; 2.01	23.3	1.91; 2.00
17	47.3		47.0		47.0		47.0		46.9	
18	47.4	3.08 (*dd*, 13.4, 4.4)	41.6	3.15 (*dd*, 13.6, 4.2)	41.6	3.15 (*dd*, 13.8, 4.0)	41.6	3.15 (*dd*, 13.6, 3.7)	41.6	3.17 (*dd*, 13.8, 4.0)
19	41.6	2.14; 2.52 (*t*, 13.6)	46.1	1.18; 1.69	46.1	1.19; 1.68	46.1	1.19; 1.70	46.1	1.20; 1.69
20	148.3		30.7		30.7		30.7		30.6	
21	30.0	2.05; 2.15	33.9	1.08; 1.30	33.9	1.08; 1.29	33.9	1.09; 1.30	33.9	1.09; 1.29
22	37.6	1.73; 2.02	32.5	1.74; 1.85	32.5	1.73; 1.85	32.5	1.75; 1.86	32.5	1.76; 1.87
23	63.9	3.75; 4.14	63.9	3.74; 4.11	63.9	3.74; 4.14	63.8	3.71; 4.13	63.9	3.73; 4.11
24	13.9	1.08 (*s*)	13.8	1.06 (*s*)	13.9	1.09 (*s*)	13.9	1.03 (*s*)	13.8	1.05 (*s*)
25	16.2	0.95 (*s*)	16.2	0.95 (*s*)	16.2	0.96 (*s*)	16.1	0.96 (*s*)	16.2	0.95 (*s*)
26	17.5	1.09 (*s*)	17.5	1.10 (*s*)	17.5	1.10 (*s*)	17.5	1.11 (*s*)	17.5	1.11 (*s*)
27	25.9	1.13 (*s*)	26.0	1.15 (*s*)	26.0	1.16 (*s*)	26.0	1.16 (*s*)	25.9	1.16 (*s*)
28	175.7		176.4		176.5		176.4		176.4	
29	107.2	4.63; 4.69	33.0	0.85 (*s*)	33.0	0.85 (*s*)	33.0	0.85 (*s*)	33.0	0.84 (*s*)
30			23.6	0.87 (*s*)	23.6	0.86 (*s*)	23.6	0.87 (*s*)	23.6	0.86 (*s*)
28 Sugar chain										
	28-*O*-*Glc*		28-*O*-*Glc*		28-*O*-*Glc*		28-*O*-*Glc*		28-*O*-*Glc*	
1	95.7	6.20 (*d*, 8.8)	95.6	6.23 (*d*, 7.8)	95.6	6.22 (*d*, 8.0)	95.6	6.23 (*d*, 7.9)	95.6	6.25 (*d*, 8.1)
2	73.8	4.10	73.8	4.13	73.8	4.11	73.8	4.12	73.9	4.13
3	78.6	4.19	78.7	4.20	78.7	4.20	78.7	4.20	78.7	4.20

4	70.9	4.29	70.8	4.29	70.8	4.28	70.8	4.28	70.9	4.32
5	77.8	4.09	78.0	4.10	78.0	4.10	78.0	4.10	77.9	4.12
6	69.5	4.33; 4.68	69.2	4.32; 4.65	69.2	4.31; 4.65	69.2	4.32; 4.66	69.4	4.35; 4.70
	28-*O*-Glc-6-*Glc*		28-*O*-Glc-6-*Glc*		28-*O*-Glc-6-*Glc*		28-*O*-Glc-6-*Glc*		28-*O*-Glc-6-*Glc*	
1	105.3	5.00 (*d*, 7.8)	104.8	4.98 (*d*, 7.2)	104.8	4.98 (*d*, 7.9)	104.8	4.98 (*d*, 7.6)	105.2	5.03 (*d*, 7.7)
2	75.1	3.99 (*t*, 8.0)	75.3	3.93 (*t*, 8.6)	75.3	3.92 (*t*, 8.0)	75.3	3.93 (*t*, 8.4)	75.1	3.99 (*t*, 8.2)
3	78.4[a]	4.19	76.5	4.14	76.5	4.15	76.4	4.14	78.4[a]	4.20
4	71.5	4.20	78.2	4.40	78.2	4.40	78.2	4.41	71.4	4.20
5	78.3[a]	3.88	77.1	3.66	77.1	3.65	77.1	3.66	78.3[a]	3.88
6	62.6	4.36; 4.47	61.2	4.08; 4.20	61.2	4.09; 4.20	61.2	4.09; 4.20	62.6	4.35; 4.47
			28-*O*-Glc-6-Glc-4-*Rha*		28-*O*-Glc-6-Glc-4-*Rha*		28-*O*-Glc-6-Glc-4-*Rha*			
1			102.7	5.84 (*br. s*)	102.7	5.83 (*br. s*)	102.7	5.84 (*br. s*)		
2			72.5	4.66	72.5	4.66	72.5	4.66		
3			72.7	4.53	72.7	4.53 (*dd*, 9.2, 3.1)	72.7	4.53		
4			73.9	4.31	73.9	4.31	73.9	4.31		
5			70.2	4.95	70.2	4.94	70.2	4.95		
6			18.5	1.69 (*d*, 6.3)	18.5	1.68 (*d*, 6.0)	18.4	1.69 (*d*, 6.2)		
3 Sugar chain										
	3-*O*-*Ara*		3-*O*-*Ara*		3-*O*-*Ara*		3-*O*-*Ara*		3-*O*-*Ara*	
1	104.1	5.13 (*d*, 5.8)	103.9	5.15 (*d*, 5.8)	104.2	5.13 (*d*, 6.1)	104.4	5.03 (*d*, 6.5)	103.9	5.15 (*d*, 5.7)
2	74.5[b]	4.66	74.4	4.64	74.4	4.67	76.4	4.47	74.5	4.64
3	82.5	4.07	81.2	4.13	82.5	4.07	75.1	4.00	81.1	4.13
4	68.8	4.38	68.4	4.40	68.8	4.38	69.5	4.11	68.4	4.40
5	65.5	3.70; 4.26	65.2	3.69; 4.25	65.5	3.69; 4.26	66.1	3.65; 4.21	65.2	3.68; 4.25
	3-*O*-Ara-2-*Rha*		3-*O*-Ara-2-*Rha*		3-*O*-Ara-2-*Rha*		3-*O*-Ara-2-*Rha*		3-*O*-Ara-2-*Rha*	
1	101.8	6.18 (*br. s*)	101.9	6.09 (*br. s*)	101.8	6.19 (*br. s*)	100.7	6.44 (*br. s*)	101.9	6.09 (*br. s*)
2	72.3	4.71	72.3	4.70	72.3	4.70	82.4	4.72 (*br. d*, 3.3)	72.3	4.69
3	72.5	4.58	72.6	4.57	72.5	4.58 (*dd*, 9.4, 3.2)	72.5	4.60	72.6	4.57 (*dd*, 9.0, 3.2)
4	73.9	4.25	73.9	4.24	73.9	4.25	74.5	4.10	73.9	4.25
5	69.9	4.68	70.0	4.63	69.9	4.68	69.3	4.60	70.0	4.65
6	18.5	1.64 (*d*, 6.1)	18.5	1.64 (*d*, 5.9)	18.5	1.64 (*d*, 6.0)	18.4	1.56 (*d*, 6.1)	18.5	1.64 (*d*, 6.1)
	3-*O*-Ara-3-*Xyl*		3-*O*-Ara-3-*Ara*		3-*O*-Ara-3-*Xyl*		3-*O*-Ara-2-Rha-2-*Gal*		3-*O*-Ara-3-*Ara*	
1	105.5	4.94 (*d*, 7.3)	104.8	4.94 (*d*, 6.1)	105.5	4.92 (*d*, 7.4)	107.9	5.14 (*d*, 7.8)	104.8	4.97 (*d*, 6.6)
2	74.4[b]	3.93 (*t*, 7.9)	72.2	4.46 (*t*, 7.8)	74.5	3.92 (*t*, 8.0)	73.2	4.50	72.2	4.47
3	77.8	4.09	74.0	4.13	77.8	4.10	75.3	4.10	74.0	4.13
4	70.8	4.10	68.8	4.34	70.8	4.10	70.3	4.45	68.8	4.35
5	67.0	3.64; 4.30	66.4	3.76; 4.33	67.0	3.63; 4.28	77.2	3.99	66.4	3.76; 4.33
6							62.3	4.29; 4.38		

NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 1; +ESI-IT-MS *m/z*: 1373 $[M+Na]^+$; +ESI-IT-MS^2 (1373) *m/z*: 903 $[M+Na-470]^+$, 859 $[M+Na-470-44]^+$; +ESI-IT-MS^3 (1373–903) *m/z*: 859 $[(M+Na-470)-44]^+$, 741 $[(M+Na-470)-162]^+$, 697 $[(M+Na-470)-162-44]^+$; -ESI-IT-MS *m/z*: 1349 $[M-H]^-$; -ESI-IT-MS^2 (1349) *m/z*: 879 $[M-H-470]^-$, 717 $[M-H-470-162]^-$; -ESI-IT-MS^3 (1349–879) *m/z*: 835 $[(M-H-470)-44]^-$, 717 $[(M-H-470)-162]^-$, 699 $[(M-H-470)-162-18]^-$, 571 $[(M-H-470)-162-146]^-$, 553 $[(M-H-470)-162-146-18]^-$, 439 $[(M-H-470)-162-146-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1373.6335 $[M+Na]^+$ (calcd. for $C_{64}H_{102}NaO_{30}$, 1373.6354).

3.3.3. 3-O-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-30-norhederagenin-28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**3**)

White amorphous powder; $[\alpha]_D^{27}$ 9.9 (c 0.13, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3389, 2937, 1743, 1650, 1449, 1389, 1065; for ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 1; +ESI-IT-MS *m/z*: 1359 $[M+Na]^+$; +ESI-IT-MS^2 (1359) *m/z*: 889 $[M+Na-470]^+$, 743 $[M+Na-470-146]^+$, 493 $[470+Na]^+$; +ESI-IT-MS^3 (1359-889) *m/z*: 845 $[(M+Na-470)-44]^+$, 757 $[(M+Na-470)-132]^+$, 743 $[(M+Na-470)-146]^+$, 713 $[(M+Na-470)-132-44]^+$, 699 $[(M+Na-470)-146-44]^+$; +ESI-IT-MS^3 (1359-493) *m/z*: 475, 447, 421, 405, 349, 347, 331, 289, 203; -ESI-IT-MS *m/z*: 1335 $[M-H]^-$; -ESI-IT-MS^2 (1335) *m/z*: 865 $[M-H-470]^-$, 733 $[M-H-470-132]^-$, 719 $[M-H-470-146]^-$; -ESI-IT-MS^3 (1335-865) *m/z*: 733 $[(M-H-470)-132]^-$, 719 $[(M-H-470)-146]^-$, 587 $[(M-H-470)-132-146]^-$, 569 $[(M-H-470)-132-146-18]^-$, 455 $[(M-H-470)-132-146-132]^-$; -ESI-IT-MS^3 (1335-733) *m/z*: 587 $[(M-H-470-132)-146]^-$, 569 $[(M-H-470-132)-146-18]^-$, 455 $[(M-H-470-132)-146-132]^-$; -ESI-IT-MS^3 (1335-719) *m/z*: 587 $[(M-H-470-146)-132]^-$, 569 $[(M-H-470-146)-132-18]^-$, 455 $[(M-H-470-146)-132-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1359.6134 $[M+Na]^+$ (calcd. for $C_{63}H_{100}NaO_{30}$, 1359.6197).

3.3.4. 3-O-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-30-norhederagenin-28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**4**)

White amorphous powder; $[\alpha]_D^{28}$ 13.7 (c 0.12, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3401, 2938, 1739, 1642, 1447, 1381, 1065; for ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 1; +ESI-IT-MS *m/z*: 1359 $[M+Na]^+$; +ESI-IT-MS^2 (1359) *m/z*: 889 $[M+Na-470]^+$, 845 $[M+Na-470-44]^+$, 743 $[M+Na-470-146]^+$, 493 $[470+Na]^+$; +ESI-IT-MS^3 (1359-889) *m/z*: 845 $[(M+Na-470)-44]^+$, 757 $[(M+Na-470)-132]^+$, 743 $[(M+Na-470)-146]^+$, 713 $[(M+Na-470)-132-44]^+$, 699 $[(M+Na-470)-146-44]^+$, 611 $[(M+Na-470)-146-132]^+$; +ESI-IT-MS^3 (1359-493) *m/z*: 475, 447, 421, 405, 349, 347, 331, 289, 203; -ESI-IT-MS *m/z*: 1335 $[M-H]^-$; -ESI-IT-MS^2 (1335) *m/z*: 865 $[M-H-470]^-$, 733 $[M-H-470-132]^-$, 719 $[M-H-470-146]^-$; -ESI-IT-MS^3 (1335-865) *m/z*: 733 $[(M-H-470)-132]^-$, 719 $[(M-H-470)-146]^-$, 587 $[(M-H-470)-132-146]^-$, 569 $[(M-H-470)-132-146-18]^-$; -ESI-IT-MS^3 (1335-733) *m/z*: 587 $[(M-H-470-132)-146]^-$, 569 $[(M-H-470-132)-146-18]^-$, 455 $[(M-H-470-132)-146-132]^-$; -ESI-IT-MS^3 (1335-719) *m/z*: 587 $[(M-H-470-146)-132]^-$, 569 $[(M-H-470-146)-132-18]^-$, 455 $[(M-H-470-146)-132-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1359.6147 $[M+Na]^+$ (calcd. for $C_{63}H_{100}NaO_{30}$, 1359.6197).

3.3.5. 3-O-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-30-norhederagenin-28-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**5**)

White amorphous powder; $[\alpha]_D^{27}$ 17.4 (c 0.10, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3451, 2930, 1739, 1653, 1454, 1388, 1072; for ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 1; +ESI-IT-MS *m/z*: 1213 $[M+Na]^+$; +ESI-IT-MS^2 (1213) *m/z*: 889 $[M+Na-324]^+$, 743 $[M+Na-324-146]^+$; +ESI-IT-MS^3 (1213-889) *m/z*: 845 $[(M+Na-324)-44]^+$, 757 $[(M+Na-324)-132]^+$, 743 $[(M+Na-324)-146]^+$, 713 $[(M+Na-324)-132-44]^+$, 699 $[(M+Na-324)-146-44]^+$, 611 $[(M+Na-324)-146-132]^+$; -ESI-IT-MS *m/z*: 1189 $[M-H]^-$; -ESI-IT-MS^2 (1189) *m/z*: 865 $[M-H-324]^-$, 733 $[M-H-324-132]^-$, 719 $[M-H-324-146]^-$; -ESI-IT-MS^3 (1189–865) *m/z*: 733 $[(M-H-324)-132]^-$, 719 $[(M-H-324)-146]^-$, 587 $[(M-H-324)-132-146]^-$, 569 $[(M-H-324)-132-146-18]^-$, 455 $[(M-H-324)-132-146-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1213.5597 $[M+Na]^+$ (calcd. for $C_{57}H_{90}NaO_{26}$, 1213.5618).

3.3.6. 3-O-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-30-norhederagenin-28-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**6**)

White amorphous powder; $[\alpha]_D^{27}$ 10.2 (c 0.10, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3451, 2932, 1743, 1656, 1455, 1381, 1071; for ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 2; +ESI-IT-MS *m/z*: 1213 $[M+Na]^+$; +ESI-IT-MS^2 (1213) *m/z*: 889 $[M+Na-324]^+$, 743 $[M+Na-324-146]^+$; +ESI-IT-MS^3 (1213–889) *m/z*: 845 $[(M+Na-324)-44]^+$, 757 $[(M+Na-324)-132]^+$, 743 $[(M+Na-324)-146]^+$, 713 $[(M+Na-324)-132-44]^+$, 699 $[(M+Na-324)-146-44]^+$, 611 $[(M+Na-324)-146-132]^+$; -ESI-IT-MS *m/z*: 1189 $[M-H]^-$; -ESI-IT-MS^2 (1189) *m/z*: 865 $[M-H-324]^-$, 733 $[M-H-324-132]^-$; -ESI-IT-MS^3 (1189-865) *m/z*: 733 $[(M-H-324)-132]^-$, 719 $[(M-H-324)-146]^-$, 587 $[(M-H-324)-132-146]^-$, 569 $[(M-H-324)-132-146-18]^-$, 455 $[(M-H-324)-132-146-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1213.5584 $[M+Na]^+$ (calcd. for $C_{57}H_{90}NaO_{26}$, 1213.5618).

3.3.7. 3-O-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-hederagenin-28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**7**)

White amorphous powder; $[\alpha]_D^{27}$ −6.6 (c 0.13, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3322, 2936, 1740, 1648, 1456, 1383, 1066; for ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 2; +ESI-IT-MS *m/z*: 1375 $[M+Na]^+$; +ESI-IT-MS^2 (1375) *m/z*: 905 $[M+Na-470]^+$, 493 $[470+Na]^+$; +ESI-IT-MS^3 (1375-905) *m/z*: 861 $[(M+Na-470)-44]^+$, 773 $[(M+Na-470)-132]^+$, 759 $[(M+Na-470)-146]^+$, 729 $[(M+Na-470)-132-44]^+$, 715 $[(M+Na-470)-146-44]^+$, 627 $[(M+Na-470)-146-132]^+$; +ESI-IT-MS^3 (1375-493) *m/z*: 475, 447, 421, 405, 349, 347, 331, 289, 203; -ESI-IT-MS *m/z*: 1351 $[M-H]^-$; -ESI-IT-MS^2 (1351) *m/z*: 881 $[M-H-470]^-$, 749 $[M-H-470-132]^-$, 735 $[M-H-470-146]^-$; -ESI-IT-MS^3 (1351-881) *m/z*: 749 $[(M-H-470)-132]^-$, 735 $[(M-H-470)-146]^-$, 603 $[(M-H-470)-132-146]^-$, 585 $[(M-H-470)-132-146-18]^-$, 471 $[(M-H-470)-132-146-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1375.6485 $[M+Na]^+$ (calcd. for $C_{64}H_{104}NaO_{30}$, 1375.6510).

3.3.8. 3-O-β-D-xylopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-hederagenin-28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**8**)

White amorphous powder; $[\alpha]_D^{27}$ −6.2 (c 0.10, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3365, 2939, 1736, 1642, 1457, 1388, 1058; for ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 2; +ESI-IT-MS *m/z*: 1375 $[M+Na]^+$; +ESI-IT-MS^2 (1375) *m/z*: 905 $[M+Na-470]^+$, 759 $[M+Na-470-146]^+$, 493 $[470+Na]^+$; +ESI-IT-MS^3 (1375-905) *m/z*: 861 $[(M+Na-470)-44]^+$, 773 $[(M+Na-470)-132]^+$, 759 $[(M+Na-470)-146]^+$, 729 $[(M+Na-470)-132-44]^+$, 715 $[(M+Na-470)-146-44]^+$, 627 $[(M+Na-470)-146-132]^+$; +ESI-IT-MS^3 (1375-

493) *m/z*: 475, 447, 421, 405, 349, 347, 331, 289, 203; -ESI-IT-MS *m/z*: 1351 $[M-H]^-$; -ESI-IT-MS^2 (1351) *m/z*: 881 $[M-H-470]^-$, 749 $[M-H-470-132]^-$, 735 $[M-H-470-146]^-$, 603 $[M-H-470-132-146]^-$, 585 $[M-H-470-132-146-18]^-$, 471 $[M-H-470-132-146-132]^-$; -ESI-IT-MS^3 (1351-881) *m/z*: 749 $[(M-H-470)-132]^-$, 735 $[(M-H-470)-146]^-$, 603 $[(M-H-470)-132-146]^-$, 585 $[(M-H-470)-132-146-18]^-$, 471 $[(M-H-470)-132-146-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1375.6521 $[M+Na]^+$ (calcd. for $C_{64}H_{104}NaO_{30}$, 1375.6510).

3.3.9. 3-O-β-D-galactopyranosyl-(1 → 2)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-hederagenin-28-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**9**)

White amorphous powder; $[\alpha]_D^{27}$ −2.0 (c 0.43, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3388, 2940, 1745, 1641, 1456, 1389, 1076; for ^1H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 2; +ESI-IT-MS *m/z*: 1405 $[M+Na]^+$; +ESI-IT-MS^2 (1405) *m/z*: 935 $[M+Na-470]^+$, 773 $[M+Na-470-162]^+$; +ESI-IT-MS^3 (1405-935) *m/z*: 891 $[(M+Na-470)-44]^+$, 773 $[(M+Na-470)-162]^+$, 729 $[(M+Na-470)-162-44]^+$, 627 $[(M+Na-470)-162-146]^+$, 583 $[(M+Na-470)-162-146-44]^+$; -ESI-IT-MS *m/z*: 1381 $[M-H]^-$; -ESI-IT-MS^2 (1381) *m/z*: 911 $[M-H-470]^-$, 749 $[M-H-470-162]^-$, 603 $[M-H-470-162-146]^-$; -ESI-IT-MS^3 (1381-911) *m/z*: 749 $[(M-H-470)-162]^-$, 603 $[(M-H-470)-162-146]^-$, 585 $[(M-H-470)-162-146-18]^-$, 471 $[(M-H-470)-162-146-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1405.6645 $[M+Na]^+$ (calcd. for $C_{65}H_{106}NaO_{31}$, 1405.6616).

3.3.10. 3-O-α-L-arabinopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-hederagenin-28-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**12**)

White amorphous powder; $[\alpha]_D^{27}$ 2.7 (c 0.15, MeOH–H_2O 1:1); IR (KBr) ν_{max} (cm^{-1}) 3401, 2928, 1739, 1649, 1457, 1385, 1074; for ^1H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz) spectroscopic data, see Table 2; +ESI-IT-MS *m/z*: 1229 $[M+Na]^+$; +ESI-IT-MS^2 (1229) *m/z*: 905 $[M+Na-324]^+$, 773 $[M+Na-324-132]^+$, 759 $[M+Na-324-146]^+$; +ESI-IT-MS^3 (1229−905) *m/z*: 861 $[(M+Na-324)-44]^+$, 773 $[(M+Na-324)-132]^+$, 759 $[(M+Na-324)-146]^+$, 729 $[(M+Na-324)-132-44]^+$, 715 $[(M+Na-324)-146-44]^+$; -ESI-IT-MS *m/z*: 1205 $[M-H]^-$; -ESI-IT-MS^2 (1205) *m/z*: 881 $[M-H-324]^-$, 749 $[M-H-324-132]^-$, 735 $[M-H-324-146]^-$; -ESI-IT-MS^3 (1205-881) *m/z*: 749 $[(M-H-324)-132]^-$, 735 $[(M-H-324)-146]^-$, 603 $[(M-H-324)-132-146]^-$, 585 $[(M-H-324)-132-146-18]^-$, 471$[(M-H-324)-132-146-132]^-$; +HR-ESI-Q-TOF-MS *m/z*: 1224.6321 $[M+NH_4]^+$ (calcd. for $C_{58}H_{98}NO_{26}$, 1224.6377).

3.4. Acid hydrolysis and derivatization of 1–9, 12 and GC analysis

Each saponin (2 mg) was hydrolyzed by heating in an ampule with a 12% HCl aqueous solution (5 mL) at 90 °C for 2 h. After extracting each reaction mixture with $CHCl_3$, the aqueous residues were individually evaporated under reduced pressure. Then, dry pyridine (1 mL) and L-cysteine methyl ester hydrochloride (2 mg) (Sigma) were added to each dry residue, with the mixture heated at 60 °C for 2 h. Each reaction mixture was next concentrated to dryness under N_2, trimethylsilyl imidazole (Fluka) was added to each residue, with the preparations individually heated at 60 °C for 1 h. The resulting solutions were extracted with cyclohexane, with each combined organic phase analyzed by GC analysis by comparison with derivatives prepared by the same reaction for standard monosaccharides (D-glucose, D-galactose, L-arabinose, D-xylose, L-rhamnose) (Sigma). Gas chromatography was performed on a HP-1701 column (0.25 mm × 30 m) using a FID detector with N_2 as carrier gas. The injector temperature was set at 250 °C and the column temperature program was as follows: the initial temperature of 200 °C was held constant for 5 min and then increased by 5 °C/min to the final temperature of 250 °C. The detector temperature was set at 280 °C.

3.5. Acid hydrolysis of the saponin-enriched fraction and preparation of hederagenin

The saponin-enriched fraction (C-M-W 60:40:8) (300 mg) was hydrolyzed by the afore-mentioned method. The aglycones so obtained were extracted with $CHCl_3$. The $CHCl_3$ extract was subjected to silica gel CC, eluted with cyclohexane-EtoAc (70:30) followed by Sephadex LH-20 CC with $CHCl_3$–MeOH (1:1) as eluent to give coarse crystals. Recrystallization of the coarse crystals in methanol yielded hederagenin (9.1 mg).

3.6. Bioassay

3.6.1. Cell culture

Mouse monocyte-macrophages RAW 264.7 (ATCC TIB-71) were maintained in RPMI 1640 medium (Gibco) supplemented with penicillin (100 U/mL) (Gibco), streptomycin (100 μg/mL) (Gibco) and 10% heat inactivated fetal bovine serum (Gibco) at 37 °C in a humidified incubator with 5% CO_2 and 95% air. The medium was routinely changed every 2 days. RAW 264.7 cells were treated by trypsinization until they attained confluence when they were used for assays during the exponential growth phase.

3.6.2. Nitric oxide analysis and cell viability assay

RAW 264.7 cells were cultured in 96-well plates at the initial density of 5×10^5 cells/mL in RPMI 1640 medium (200 μL/well) for 1 h. Then the test compound (DMSO-dissolved) at various concentrations was added (0.4 μL/well) and lipopolysaccharide (LPS) (Sigma) was also added to a final concentration of 1 μg/mL. LPS groups received LPS but not the test compound, which gave 0% for the inhibition ratio of nitric oxide-release. Control groups received 0.4 μL DMSO per well without LPS and the test compound, which gave 100% for the inhibition ratio of nitric oxide-release. Cells in the 96-well plates were then cultured for 24 h and for nitric oxide analysis. NO was determined by measuring the amount of nitrite in the cell culture supernatant, using Griess reagent (mixture of equal amount of 1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride in H_2O). Hundred microliters of the cell culture supernatant was taken from each well, briefly centrifuged (1000×*g*, 4 °C, 3 min) and mixed with 100 μL of Griess reagent, followed by incubation for 10 min at room temperature (light protected). The absorbance was measured using a microplate reader at 540 nm and the amount of nitrite calculated according to a standard calibration curve prepared from sodium nitrite (Lee et al., 1999).

The mitochondrial-dependent reduction of 3-(4,5-dimethylthizaol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan was used to measure cell respiration as an indicator of cell viability (Denizot and Lang, 1986). After 100 μL of the cell culture supernatant was taken from each well for nitric oxide analysis, MTT (Sigma) was added to each well to give a final concentration of 200 μg/mL. Cells were then incubated for 4 h. After brief centrifugalization, DMSO (100 μL) was added to each well to dissolve the formazan. The absorbance was measured using a microplate reader at 570 nm. LPS groups were considered as 100% of viable cells. The results were expressed as percentage of viable cells compared with LPS groups.

3.6.3. Measurement of cytokine (TNF-α and IL-6)

RAW 264.7 cells were cultured in 96-well plates at the initial density of 5×10^5 cells/mL in RPMI 1640 medium (200 μL/well)

for 1 h. Then the test groups, LPS groups which gave 0% for the inhibition ratio of cytokine-release, and control groups which gave 100% for the inhibition ratio of cytokine-release were treated in the same way as for NO analysis, respectively. Then, cells in the 96-well plates were cultured for 6 h and used to measure cytokine. TNF-α and IL-6 were detected in the cell culture supernatant, using respective enzyme-linked immunosorbent assay kits (mouse TNF-α ELISA kit and mouse IL-6 ELISA kit) (R&D), according to the respective manufacturer's recommendations.

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