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# Oleanolic glycosides from *Pometia ridleyi*

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**Abstract**

Six triterpenoid saponins were isolated from the stem bark of *Pometia ridleyi* along with two known saponins, acutoside A and calenduloside C. Their structures were established using one- and two-dimensional NMR and mass spectrometry as 3-*O*-β-D-apiofuranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-, 3-*O*-β-D-apiofuranosyl-(1→3)-α-L-arabinopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-, 3-*O*-β-D-apiofuranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-, 3-*O*-α-L-arabinopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-, 3-*O*-β-D-galactopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-, 3-*O*-β-D-apiofuranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-oleanolic acid. The EtOH and EtOAc extracts of the stem bark showed no cytotoxic activity. At a concentration of 23 μg/ml, the saponin mixture showed haemolytic activity and caused 50% haemolysis of a 10% suspension of sheep erythrocytes.


*Keywords:* *Pometia ridleyi*; Sapindaceae; Triterpenoid saponins; Oleanolic acid; Haemolysis

## 1. Introduction

In a continuation of our study on saponin constituents of plants of the Sapindaceae family, we have examined the stem bark of *Pometia ridleyi* King emend. Radlk. The genus *Pometia* consists of 10 species distributed from Malaysia to India (Jayasinghe et al., 2000). Two species have been studied previously, *P. pinnata* (Vijaya and Croft, 1980) and *P. eximia* (Jayasinghe et al., 1995). *P. pinnata* is used medicinally in the South Pacific Kingdom of Tonga to treat mouth infections, abdominal ailments, unclosed fontanel, obstetric and gynaecological complaints (Whistler, 1991; Singh et al., 1984). Saponins from *P. eximia*, growing in Sri Lanka, showed a variety of molluscicidal, insecticidal, antifungal, larvicidal and antibacterial activities (Jayasinghe et al., 1998, 2000; Jayasinghe and Fujimoto, 1999).

The stem bark of *P. ridleyi* was collected in Gua Musang in Malaysia. This work is a part of a collaborative research program between the CNRS (ICSN) France and the University of Malaya (Dept. of Chemistry) Malaysia (Teo et al., 1990). This species was selected in a screening program for potential cytotoxic compounds from plants growing in Malaysia. The ethanolic and ethyl acetate extracts of the stem bark were tested *in vitro* against P388 cells, topoisomerase I and tubulin, but showed no cytotoxic activity. This paper reports on the isolation and the structural elucidation of six new monodesmosidic saponins, possessing oleanolic acid as aglycone, along with two known saponins, acutoside A and calenduloside C previously isolated from *Ecballium elaterium* (Chakravarty et al., 1997). The haemolytic activity of the saponin mixture was tested and showed an activity similar to the reference saponin Merck®.

## 2. Results and discussion

The dried and powdered stem bark of *P. ridleyi* was extracted with boiling 80% methanol. The aqueous methanolic extract was concentrated and poured into acetone to give saponin mixture A. The filtrate was concentrated and again precipitated in diethyl ether to

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give saponin mixture B. Each crude saponin precipitate was dialysed, and then chromatographed on a silica gel column. Purification by a series of column chromatography over silica gel and reverse phase C-18 and finally by HPLC (High Pressure Liquid Chromatography) over C-18 or by preparative TLC afforded eight saponins (**1**–**8**). Saponins **1**, **4**, **5** and **7** were obtained from the purification of the acetone saponin mixture (A), and saponins **2**, **3**, **5**, **6** and **8** were isolated from the diethyl ether saponin mixture (B). Acid hydrolysis of the saponin mixture B gave two sapogenins, identified by TLC by comparison with authentic samples as oleanolic acid, the major component, and hederagenin. The sugars were identified as D-glucose, D-galactose, and L-arabinose by TLC and their absolute configurations were determined by measurement of the optical rotation after separation by prep. TLC. Oleanolic acid was identified as the aglycone moiety of all compounds **1**–**8** on the basis of the $^{1}H$ NMR, $^{13}C$ NMR, COSY, ROESY, HSQC, and HMBC spectra (Mahato and Kundu, 1994; Bedir et al., 2000) (Tables 1 and 2).

Saponin **1**, molecular formula $C_{42}H_{68}O_{13}$ (ESI-MS$^{-}$: $m/z$ 779 $[M-H]^{-}$), and saponin **4**, molecular formula $C_{48}H_{78}O_{18}$ (ESI-MS$^{-}$: $m/z$ 941 $[M-H]^{-}$), were identified as acutoside A, 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-oleanolic acid, and calenduloside C or elateroside B, 3-*O*-β-D-galactopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-oleanolic acid, respectively, on the basis of their spectral data. Compound **1** was previously isolated from *Passiflora quadrangularis* (Orsini et al., 1987), *Luffa acutangulata* (Nagao et al., 1991), *Ecballium elaterium* (Chakravarty et al., 1997), *Hedera helix* (Bedir et al., 2000) and *Swartia schomburkii* (Abdel-Kader et al., 2000), while compound **4** was obtained from *Calendula officinalis*, *C. arvensis* (Vidal-Ollivier et al., 1989) and *Ecballium elaterium* (Chakravarty and al., 1997).

The positive ESI-MS of compound **2** gave a quasi-molecular ion peak at $m/z$ 935 $[M+Na]^{+}$ while, in the negative mode, a quasi-molecular ion was detected at $m/z$ 911 $[M-H]^{-}$ indicating a $M_r$ of 912 amu in agree-

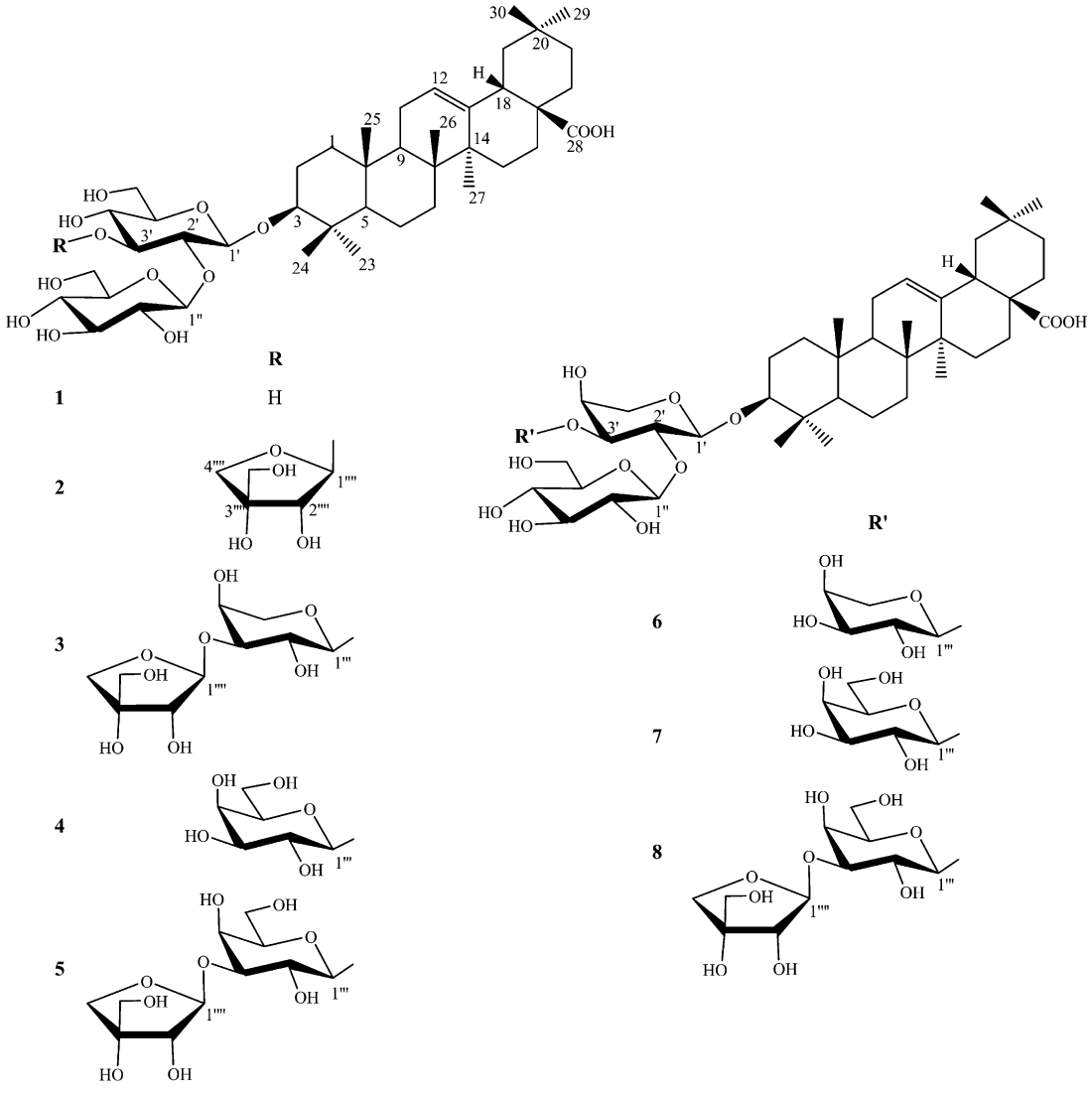

Table 1
$^{1}H$ and $^{13}C$ NMR data of saponins **2**, **3** and **5** in $CD_3OD$

| | **2** | | | **3** | | | **5** | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\delta_H$ | | $\delta_C$ | $\delta_H$ | | $\delta_C$ | $\delta_H$ | | $\delta_C$ |
| *β-D-Glucose* | | | | | | | | | |
| 1′ | 4.50 | *d* (7.8) | 103.9 | 4.50 | *d* (7.7) | 104.1 | 4.50 | *d* (7.1) | 104.1 |
| 2′ | 3.72 | *dd* (8.9, 8.2) | 76.7 | 3.78 | *dd* (8.3–7.7) | 77.0 | 3.78 | *m* | 77.1 |
| 3′ | 3.63 | *t* (8.8) | 87.5 | 3.73 | *t* (8.3) | 86.0 | 3.77 | *m* | 85.9 |
| 4′ | 3.36 | *t* (8.8) | 68.7 | 3.38 | *t* (8.5) | 68.5 | 3.41 | *t* (9.2) | 68.7 |
| 5′ | 3.31 | *m* | 76.0 | 3.32 | *m* | 76.1 | 3.35 | *m* | 76.0 |
| 6a′ | 3.68 | *dd* (11.4, 5.5) | 61.2 | 3.68 | *dd* (11.7, 5.5) | 61.3 | 3.69 | *dd* (12, 7.6) | 61.3 |
| 6b′ | 3.88 | *dd* (11.4, 2.1) | | 3.88 | *d* (11.7) | | 3.89 | *dd* (12, 1.6) | |
| *β-D-Glucose′* | | | | | | | | | |
| 1″ | 4.81 | *d* (7.8) | 102.1 | 5.01 | *d* (7.8) | 101.6 | 5.00 | *d* (7.8) | 101.7 |
| 2″ | 3.19 | *dd* (9.2, 8) | 74.5 | 3.16 | *dd* (9.2, 8.0) | 74.8 | 3.16 | *dd* (7.8, 8.6) | 74.7 |
| 3″ | 3.37 | *t* (9.2) | 76.5 | 3.36 | *t* (9.2) | 76.8 | 3.37 | *t* (9) | 76.8 |
| 4″ | 3.10 | *t* (9.3) | 71.0 | 3.09 | *t* (9.2) | 71.0 | 3.09 | *t* (9.3) | 71.0 |
| 5″ | 3.29 | *m* | 77.1 | 3.31 | *ddd* (9.2, 8.0, 1.8) | 77.0 | 3.31 | *ddd* (9.3, 7.4, 1.8) | 77.0 |
| 6a″ | 3.55 | *dd* (12.1, 6.6) | 62.3 | 3.56 | *dd* (11.5, 8.0) | 62.2 | 3.57 | *dd* (12.3, 7.1) | 62.1 |
| 6b″ | 3.86 | *dd* (12.1, 2.1) | | 3.85 | *dd* (11.5, 1.8) | | 3.85 | *dd* (12.3, 1.8) | |
| *β-D-Galactose* | | | | | | | | | |
| 1‴ | | | | | | | 4.71 | *d* (7.8) | 102.9 |
| 2‴ | | | | | | | 3.74 | *dd* (10, 8.2) | 70.7 |
| 3‴ | | | | | | | 3.55 | *dd* (10, 3) | 81.2 |
| 4‴ | | | | | | | 4.00 | *d* (3) | 68.5 |
| 5‴ | | | | | | | 3.80 | *dd* (8, 4.7) | 75.4 |
| 6a‴ | | | | | | | 3.70 | *dd* (11.4, 4.7) | 61.8 |
| 6b‴ | | | | | | | 3.78 | *dd* (11.4, 8) | |
| *α-L-Arabinose* | | | | | | | | | |
| 1‴ | | | | 4.63 | *d* (7.7) | 103.3 | | | |
| 2‴ | | | | 3.74 | *dd* (8.8, 8.2) | 70.6 | | | |
| 3‴ | | | | 3.57 | *dd* (8.8, 3.3) | 80.7 | | | |
| 4‴ | | | | 3.98 | *m* | 68.4 | | | |
| 5a‴ | | | | 3.93 | *dd* (12.4, 1.4) | 66.1 | | | |
| 5b‴ | | | | 3.65 | *brd* (12.4) | | | | |
| *β-D-Apiose* | | | | | | | | | |
| 1⁗ | 5.29 | *d* (4.2) | 110.1 | 5.23 | *d* (2.7) | 110.7 | 5.22 | *d* (2.7) | 110.7 |
| 2⁗ | 4.08 | *d* (4.2) | 76.2 | 4.06 | *d* (2.7) | 76.7 | 4.06 | *d* (2.7) | 76.7 |
| 3⁗ | | | 78.9 | – | | 78.9 | – | | 78.9 |
| 4a⁗ | 4.18 | *d* (9.6) | 73.3 | 4.11 | *d* (9.7) | 73.6 | 4.10 | *d* (9.7) | 73.6 |
| 4b⁗ | 3.82 | *d* (9.6) | | 3.79 | *d* (9.7) | | 3.79 | *d* (9.7) | |
| 5a/b⁗ | 3.56, 3.60 | *d* (11.2) *d* (11.2) | 62.8 | 3.63 | *s* | 63.8 | 3.63 | *s* | 63.7 |
| *Oleanolic acid* | | | | | | | | | |
| 3 | 3.23 | *dd* (11.4, 4.4) | 91.0 | 3.24 | *dd* (11.7, 4.2) | 90.8 | 3.24 | *dd* (11.4, 4.3) | 90.8 |
| 12 | 5.23 | *brt* ($W_{1/2}$ = 7.6) | 120.9 | 5.26 | *brt* ($W_{1/2}$ = 7.5) | 122.1 | 5.26 | *brt* ($W_{1/2}$ = 7) | 122.0 |
| 13 | – | – | 145.2 | – | | 143.8 | - | | 144.0 |
| 18 | 2.93 | *dd* (13.3, 3.1) | 42.0 | 2.88 | *dm* (12.8) | 41.3 | 2.88 | *dd* (13, 2.4) | 41.4 |
| 23 | 1.10 | *s* | 27.6 | 1.10 | *s* | 26.8 | 1.10 | *s* | 26.8 |
| 24 | 0.87 | *s* | 16.0 | 0.89 | *s* | 15.4 | 0.90 | *s* | 15.4 |
| 25 | 0.97 | *s* | 15.0 | 0.98 | *s* | 14.4 | 0.98 | *s* | 14.4 |
| 26 | 0.89 | *s* | 17.2 | 0.84 | *s* | 16.3 | 0.85 | *s* | 16.3 |
| 27 | 1.15 | *s* | 25.5 | 1.18 | *s* | 24.9 | 1.18 | *s* | 24.9 |
| 28 | – | | 178.0 | – | | 180.7 | – | | na[a] |
| 29 | 0.89 | *s* | 33.0 | 0.93 | *s* | 32.1 | 0.92 | *s* | 32.1 |
| 30 | 0.97 | *s* | 23.4 | 0.96 | *s* | 22.5 | 0.97 | *s* | 22.6 |

[a] na: not assigned.

Table 2
$^{1}H$ and $^{13}C$ NMR data of saponins **6–8** in $CD_3OD$

| | **6** | | | **7** | | | **8** | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\delta_H$ | | $\delta_C$ | $\delta_H$ | | $\delta_C$ | $\delta_H$ | | $\delta_C$ |
| *α-L-Arabinose* | | | | | | | | | |
| 1′ | 4.43 | *d* (7.6) | 104.7 | 4.42 | *d* (7.7) | 104.8 | 4.42 | *d* (7.7) | 104.7 |
| 2′ | 4.01 | *dd* (8.9, 7.8) | 75.0 | 4.00 | *dd* (9.6, 7.5) | 75.1 | 4.00 | *dd* (10.0, 7.7) | 75.1 |
| 3′ | 3.80 | *dd* (9.2, 3.1) | 82.6 | 3.84 | *brd* (9.2) | 83.5 | 3.85 | *dd* (10.0, 3.6) | 86.0 |
| 4′ | 4.03 | *m* | 68.6 | 4.09 | *m* | 68.6 | 4.09 | *m* | 68.7 |
| 5a′ | 3.88 | *dd* (12.3, 2.3) | 65.2 | 3.87 | *dd* (12.7, 2.2) | 65.2 | 3.89 | *dd* (12.6, 3) | 65.2 |
| 5b′ | 3.58 | *brd* (12.3) | | 3.59 | *dd* (12.7, 0.9) | | 3.59 | *brd* (12.6) | |
| *α-L-Arabinose′* | | | | | | | | | |
| 1″ | 4.55 | *d* (7.2) | 104.4 | | | | | | |
| 2″ | 3.66 | *dd* (9.2, 7.2) | 71.3 | | | | | | |
| 3″ | 3.52 | *dd* (9.2, 3.3) | 73.0 | | | | | | |
| 4″ | 3.83 | *m* | 68.4 | | | | | | |
| 5a″ | 3.88 | *dd* (12.3, 2.3) | 65.8 | | | | | | |
| 5b″ | 3.58 | *brd* (12.3) | | | | | | | |
| *β-D-Galactose* | | | | | | | | | |
| 1″ | | | | 4.58 | *d* (7.8) | 101.3 | 4.63 | *d* (7.8) | 104.1 |
| *2″* | | | | 3.32 | *dd* (9.2, 7.5) | 77.5 | 3.76 | *dd* (10.0, 7.8) | 70.6 |
| 3″ | | | | 3.30 | *dd* (9.3, 3.3) | 73.6 | 3.52 | *dd* (10.0, 3.0) | 81.1 |
| *4″* | | | | 3.86 | *d* (3.3) | 68.9 | 4.00 | *d* (3.0) | 68.5 |
| 5″ | | | | 3.55 | *m* | 75.3 | 3.57 | *m* | 75.0 |
| 6a″ | | | | 3.74 | *dd* (11.4, 4) | 61.1 | 3.72 | *m* | 60.9 |
| 6b″ | | | | 3.72 | *dd* (11.4, 5.5) | | 3.73 | *m* | |
| *β-D-Glucose* | | | | | | | | | |
| 1‴ | 4.86 | *d* (7.7) | 102.2 | 4.88 | *d* (7.9) | 102.3 | 4.87 | *d* (7.9) | 102.0 |
| 2‴ | 3.13 | *dd* (9.5, 7.7) | 74.6 | 3.12 | *dd* (9.2, 8) | 74.7 | 3.13 | *dd* (9.2, 8.2) | 74.7 |
| 3‴ | 3.34 | *m* | 76.9 | 3.36 | *dd* (9.3, 9.2) | 77.2 | 3.35 | *m* | 76.9 |
| 4‴ | 3.09 | *t* (9.5) | 71.0 | 3.08 | *t* (9.3) | 71.0 | 3.90 | *t* (9.3) | 71.0 |
| 5‴ | 3.31 | *ddd* (9.3, 8, 2.3) | 76.9 | 3.31 | *ddd* (9.3, 7.1, 2.2) | 77.2 | 3.30 | *m* | 76.9 |
| 6a‴ | 3.57 | *dd* (11, 8) | 62.2 | 3.56 | *dd* (11.8, 7.1) | 62.2 | 3.56 | *dd* (12.2, 7) | 62.2 |
| 6b‴ | 3.84 | *dd* (11, 2.3) | | 3.83 | *dd* (11.8, 2.2) | | 3.84 | *dd* (12.2, 3) | |
| *β-D-Apiose* | | | | | | | | | |
| 1⁗ | | | | | | | 5.22 | *d* (2.6) | 110.6 |
| 2⁗ | | | | | | | 4.05 | *d* (2.6) | 76.7 |
| 3⁗ | | | | | | | - | | 77.9 |
| 4a⁗ | | | | | | | 4.10 | *d* (9.6) | 73.6 |
| 4b⁗ | | | | | | | 3.79 | *d* (9.6) | |
| 5a/b⁗′ | | | | | | | 3.63 | *s* | 63.8 |
| *Oleanolic acid* | | | | | | | | | |
| 3 | 3.19 | *dd* (11.7, 4.5) | 90.4 | 3.20 | *dd* (11.5, 4.3) | 90.6 | 3.19 | *dd* (11.7, 4) | 90.5 |
| 12 | 5.26 | *brt* ($W_{1/2}$ = 7.5) | 122.2 | 5.25 | *brt* ($W_{1/2}$ = 7.2) | 121.6 | 5.26 | *m* | 121.5 |
| 13 | – | | 143.7 | – | | 144.2 | – | | 144.4 |
| 18 | 2.87 | *dm* (13) | 41.3 | 2.89 | *dm* (13) | 41.5 | 2.90 | *dm* (12.4) | 41.6 |
| 23 | 1.09 | *s* | 26.8 | 1.09 | *s* | 26.7 | 1.09 | *s* | 26.8 |
| 24 | 0.89 | *s* | 15.3 | 0.89 | *s* | 15.3 | 0.89 | *s* | 15.3 |
| 25 | 0.98 | *s* | 14.4 | 0.97 | *s* | 14.4 | 0.98 | *s* | 14.4 |
| 26 | 0.84 | *s* | 16.2 | 0.86 | *s* | 16.4 | 0.87 | *s* | 16.5 |
| 27 | 1.18 | *s* | 24.9 | 1.17 | *s* | 24.9 | 1.18 | *s* | 24.9 |
| 28 | – | | 180.3 | – | | na[a] | – | | 177.5 |
| 29 | 0.93 | *s* | 32.1 | 0.92 | *s* | 32.2 | 0.91 | *s* | 32.3 |
| 30 | 0.96 | *s* | 22.5 | 0.97 | *s* | 22.6 | 0.98 | *s* | 22.7 |

[a] na: not assigned.

ment with a molecular formula of $C_{47}H_{76}O_{17}$. The $MS^2$ experiment of the $[M-H]^-$ ion gave negative fragments at $m/z$ 779 $[M-H-132]^-$, 749 $[M-H-162]^-$ and 617 $[M-H-132-162]^-$ attributed to the loss of a terminal pentose, a terminal hexose, and both these terminal sugars, respectively. The $MS^3$ fragmentation of the ion at $m/z$ 617 gave a product ion at $m/z$ 455 $[genin-H]^-$ corresponding to the loss of a second hexose unit and consequently suggesting that saponin **2** contained three sugar units.

Three anomeric carbons were detected at 102.1, 103.9 and 110.1 in the $^{13}C$ NMR spectrum, attached to proton doublets at δ 4.81, 4.50 and 5.29, respectively in the HSQC experiment. Complete assignments of each glycosidic proton system were achieved by analysis of COSY and TOCSY experiments. The units with anomeric protons at $\delta$ 4.50 ($J=7.8$ Hz) and 4.81 ($J=7.8$ Hz) corresponded to two hexoses with hydroxymethyl carbons at $\delta$ 61.2 and 62.3, respectively, and were identified as two β-D-glucoses (Table 1). The deshielded chemical shifts of the anomeric atoms at $\delta_H$ 5.29 and $\delta_C$ 110.1 of the third glycosidic unit indicated a furanosyl moiety, identified as an apiose with two AX spin systems at $\delta$ 5.29 and 4.08 ppm ($J_{1,2}=4.2$ Hz) and $\delta$ 4.18 and 3.82 ($J_{4,4'}=9.6$ Hz) and one AB spin system at $\delta$ 3.56 and 3.60 ($J_{5,5'}=11.2$ Hz). The β configuration of the anomeric position of the glucosyl units was deduced from the values of the coupling constant of the H-1, while the configuration of the anomeric position of the apiosyl unit was determined as β, since the $^{13}C$ NMR data were in good agreement with those reported for β-D-apiofuranosides (Jayasinghe et al., 1995; Ishi and Yanagisawa, 1998). The downfield shifts of C-2 ($\delta$ 76.7) and C-3 ($\delta$ 87.5) of the first glucosyl moiety suggested the points of linkage of the trisaccharidic chain (Table 1). The cross peaks observed in the HMBC experiment between C-3 ($\delta$ 91.0) of oleanolic acid and H-1 of glucose ($\delta$ 4.50) (glc) and between C-2 ($\delta$ 76.7) and C-3 ($\delta$ 87.5) of this inner glucose and H-1 ($\delta$ 4.81) of the terminal glucose unit (glc′) and H-1 ($\delta$ 5.29) of the terminal apiose unit, respectively, showed that the saponin **2** is 3-*O*-β-D-apiofuranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-oleanolic acid.

The negative ESI-MS of saponin **3** gave a quasi-molecular ion at $m/z$ 1043 $[M-H]^-$, indicating a molecular formula of $C_{52}H_{84}O_{21}$ ($M_r$ 1044), suggesting a supplementary pentose unit compared to saponin **2**. This hypothesis was confirmed by the $MS^2$ experiment of the $[M-H]^-$ ion which gave a first fragment at $m/z$ 911 $[M-H-132]^-$. The other fragments were detected at $m/z$ 881 $[M-H-162]^-$ and 749 $[M-H-132-162]^-$, attributed to the loss of a terminal hexose and a terminal pentose, and at $m/z$ 779 $[(M-H)-132-132]^-$ due to the loss of two successive pentoses. The $MS^3$ experiment of $[M-H-132]^-$ at $m/z$ 911 gave ion fragments at $m/z$ 779 and 749 and oleanolic acid at $m/z$ 455 $[genin-H]^-$ after the loss of a second hexose unit.

Saponin **3** was shown to contain four sugar residues from the HSQC spectrum. The anomeric proton signals at $\delta$ 4.50, 4.63, 5.01 and 5.23 gave correlations with carbon signals at $\delta$ 104.1, 103.3, 101.6 and 110.7, respectively. Evaluation of the spin–spin couplings from analysis of COSY and TOCSY experiments and the chemical shifts of the glycosidic carbons with an HSQC experiment, allowed the identification of the three sugars constituting the glycosidic part of saponin **2**: a terminal β-D-glucopyranosyl [$\delta$ 5.01 (glc′)], a 2,3-disubstituted β-D-glucopyranosyl [$\delta$ 4.50 (glc)] and a terminal β-D-apiofuranosyl ($\delta$ 5.23). The supplementary pentose was identified as an α-L-arabinopyranosyl ($\delta$ 4.63) (Table 1). A ROESY experiment revealed the attachment of the arabinose between the first glucose (glc) and the apiose of saponin **2**. Correlations are observed between $H_{glc}$-1 at $\delta$ 4.50 and H-3 of oleanolic acid, $H_{glc'}$-1 at $\delta$ 5.01 and $H_{glc}$-2 at $\delta$ 3.78, $H_{ara}$-1 at $\delta$ 4.63 and $H_{glc}$-3 at $\delta$ 3.73 and between $H_{api}$-1 at $\delta$ 5.23 and $H_{ara}$-3 at $\delta$ 3.57. Thus, saponin **3** is 3-*O*-β-D-apiofuranosyl-(1→3)-α-L-arabinopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-oleanolic acid.

The negative ESI-MS of saponin **5** gave a quasi-molecular ion at $m/z$ 1073 $[M-H]^-$, in agreement with a molecular formula of $C_{53}H_{86}O_{22}$ ($M_r$ 1074). In comparison with saponin **2**, compound **5** seems to possess a supplementary hexose unit in the glycosidic chain. The $MS^2$ experiment of the $[M-H]^-$ ion was governed by the loss of terminal sugars, a pentose and a hexose, giving fragments at $m/z$ 941 $[M-H-132]^-$, 911 $[M-H-162]^-$, and 779 $[M-H-132-162]^-$ as in saponins **2** and **3**. The $MS^3$ experiment of the ion fragment at $m/z$ 779 showed the loss of one hexose $[M-H-132-162-162]^-$ at $m/z$ 617. This fragmentation suggested that the supplementary hexose unit replaced the pentose unit found in saponin **3**.

The $^1H$ spectrum of saponin **5** showed four anomeric protons at $\delta_H$ 5.0, 4.71, 4.50 and 5.22 (Table 1). Analysis of 2D experiments (COSY, TOCSY and HSQC) permitted assignments as in saponins **2** and **3** of two β-D-glucopyranosyls with anomeric protons at $\delta$ 5.0 ($J=7.8$ Hz) (glc′) and 4.50 ($J=7.1$ Hz) (glc) and a terminal β-D-apiofuranosyl with its deshielded anomeric proton at $\delta$ 5.22 ($J=2.7$ Hz). The fourth sugar with its anomeric proton at $\delta$ 4.71 ($J=7.8$ Hz) was identified as a β-D-galactopyranosyl with the hydroxymethyl carbon at $\delta$ 61.8 and characterized by its H-4 doublet at $\delta$ 4 ($J_{3-4}=3$ Hz) in an α-equatorial position (Table 1). This sugar is substituted in position 3 since C-3 is deshielded ($\delta$ 81.2 instead of 73.7 into the known saponin **4**). Sequencing of the glycosidic part was achieved by analysis of HMBC and ROESY experiments. The HMBC spectrum showed correlations between C-3 ($\delta$ 90.8) of oleanolic acid and $H_{glc}$-1 ($\delta$ 4.50), between C-2 ($\delta$ 77.1) and C-3 (δ 85.9) of this glucose and H-1 of the second glucose ($\delta$ 5.0) and H-1 of the galactose ($\delta$ 4.71), respectively, and between C-3

(δ 81.2) of the galactose and H-1 (δ 5.22) of the apiose. Thus, the structure of saponin **5** is 3-*O*-β-D-apiofuranosyl-(1→3)-β-D-galactopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-oleanolic acid.

The ESI-MS spectra of compound **6** gave quasi-molecular ion peaks at *m/z* 905 $[M+Na]^+$ and at *m/z* 881 $[M-H]^-$, indicating a $M_r$ of 882 amu in agreement with a molecular formula of $C_{46}H_{74}O_{16}$. As with the previous compounds, the $MS^2$ experiment of the $[M-H]^-$ ion gave negative fragments attributed to the loss of terminal pentose and terminal hexose units at *m/z* 749 $[M-H-132]^-$, 719 $[M-H-162]^-$ and 587 $[M-H-132-162]^-$. The $MS^2$ experiment of the $[M+Na]^+$ ion in the positive mode gave the same fragmentation and, in addition, a fragment at *m/z* 861 $[M+Na-CO_2]^+$. The $MS^3$ fragmentation of this ion gave a positive ion at *m/z* 467 corresponding to the [trisaccharidic chain + Na]$^+$, consisting of a hexose and two pentose units.

This fact was confirmed by the detection of three anomeric signals in $^1H$ and $^{13}C$ NMR spectra at $\delta_H$ 4.43, 4.55, and 4.86 and $\delta_C$ 104.7, 104.4, and 102.2, respectively. Analysis of 2D experiments (COSY, TOCSY and HSQC) permitted the identification of a terminal β-D-glucopyranosyl ($\delta_H$ 4.86) and two α-L-arabinopyranosyls. The first arabinose was terminal with its anomeric proton at $\delta_H$ 4.55 and the second was inner and 2,3-disubstituted, as confirmed by the deshielded carbons C-2 at δ 75.0 and C-3 at δ 82.6 (Table 2). The ROESY experiment showed cross-peaks for H-1 (δ 4.43), H-2 (δ 4.01) and H-3 (δ 3.80) of the disubstituted arabinose with H-3 of oleanolic acid, H-1 of the glucose (δ 4.86) and H-1 of the terminal arabinose (δ 4.55), respectively. Thus, the structure of saponin **6** is 3-*O*-α-L-arabinopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-oleanolic acid.

The negative ESI-MS of saponin **7** gave a quasi-molecular ion at *m/z* 911 $[M-H]^-$ in agreement with a molecular formula of $C_{47}H_{76}O_{17}$ ($M_r$ 912), isomeric with saponin **2**. The $MS^2$ experiment of the $[M-H]^-$ ion gave the common negative fragment at *m/z* 749 $[M-H-162]^-$ corresponding to the loss of a terminal hexose. The $MS^3$ experiment of this ion gave fragments at *m/z* 587 $[M-H-162-162]^-$ and 455 [genin-H]$^-$ corresponding to the loss of a second hexose unit and a pentose unit, respectively. Relative to saponin **6**, the terminal pentose is replaced by a hexose in saponin **7**.

The anomeric carbons of the three sugar units were detected at δ 101.3, 102.3 and 104.8 and their corresponding proton anomeric doublets at δ 4.58, 4.18 and 4.42 (HSQC). Analysis of 2D experiments (COSY, TOCSY and HSQC) permitted the identification of an arabinose ($\delta_H$ 4.42), a terminal galactose ($\delta_H$ 4.58) and a terminal glucose ($\delta_H$ 4.88) (Table 2). HMBC correlations were detected between C-3 of oleanolic acid and H-1 of arabinose ($\delta_H$ 4.42), between C-2 ($\delta_C$ 75.1) and C-3 ($\delta_C$ 83.5) of the arabinose and H-1 of glucose and H-1 of galactose, respectively. Thus, saponin **7** is 3-*O*-β-D-galactopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-oleanolic acid.

The negative ESI-MS of saponin **8** gave a quasi-molecular ion at *m/z* 1043 $[M-H]^-$ indicating a molecular formula of $C_{52}H_{84}O_{21}$ ($M_r$ 1044), isomeric with saponin **3**. The $MS^2$ experiment of the $[M-H]^-$ ion gave common fragments at *m/z* 911, 881 and 749 for the loss of the terminal sugars. The absence of the fragment at *m/z* 779 and the $MS^3$ experiment of the ion at *m/z* 749, with fragments detected at *m/z* 587 $[M-H-132-162-162]^-$ and 455 [genin-H]$^-$, showed that in compound **8** the first unit of the glycosidic chain was a pentose disubstituted by two hexoses. The $^1H$ and $^{13}C$ NMR spectra showed the presence of four sugar residues, identified after 2D analysis (COSY, TOCSY and HSQC) as an arabinopyranosyl ($\delta_{H\text{-}1}$ 4.42; $\delta_{C\text{-}1}$ 104.7) with deshielded carbons C-2 (δ 75.1) and C-3 (δ 86.0), a terminal glucopyranosyl ($\delta_{H\text{-}1}$ 4.87 and $\delta_{C\text{-}1}$ 102.0), a terminal apiofuranosyl ($\delta_{H\text{-}1}$ 5.22 and $\delta_{C\text{-}1}$ 110.6) and a galactopyranosyl ($\delta_{H\text{-}1}$ 4.63 and $\delta_{C\text{-}1}$104.1) substituted in position 3 as attested by the deshielding of its C-3 at δ 81.1 (Table 2). As for the previous compounds, the ROESY and HMBC spectra enabled the complete assignment of the glycosidic linkages and showed that saponin **8** is 3-*O*-β-D - apiofuranosyl - (1→3)-β-D-galactopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-oleanolic acid.

The haemolytic activity of the saponin mixture B was assessed on sheep erythrocytes (10% suspension in phosphate buffer saline) using the method previously described (Voutquenne et al., 2002). The mixture of oleanolic saponins (**1**–**8**) was more active than the dialysed reference saponin mixture from Merck® (HI 30 000). 70% haemolysis was obtained at 25 μg/ml, the $HD_{100}$ was at 50 μg/ml and the $HD_{50}$ was estimated at 23 μg/ml. The quantities of isolated pure saponins were insufficient to allow us to measure the individual haemolytic activities of these compounds in order to establish structure–activity relationships.

## 3. Experimental

### 3.1. General experimental precedures

$^1H$ and $^{13}C$ NMR spectra were recorded on Bruker Avance DRX 500 ($^1H$ at 500 MHz and $^{13}C$ at 125 MHz); 2D experiments were performed using standard Bruker microprograms. ESI-MS and MS-MS experiments were recorded on a Finningan LCQ deca quadripole ion trap mass spectrometer (Finnigan MAT, San Jose, USA). The samples were introduced by direct infusion in a solution of MeOH at a flow rate of 5 μl $min^{-1}$. Optical rotations were measured in MeOH with a Perkin-Elmer 241 Polarimeter. CC were carried out on Kieselgel 60 (63–200 mesh) Merck or LiChroprep RP-18

(40–63 μm) Merck. HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, a P580 pump, a diode array detector UVD 340S (212 nm) and a Chromeleon® software. A C-18 Dionex Vydac (201SP510, 250×10 mm, 5 μm) was used for semi-preparative HPLC with a gradient of MeCN–$H_2O$ or MeOH–$H_2O$ and a flow rate of 2 ml $min^{-1}$.

### 3.2. Plant material

Stem bark of *P. ridleyi* was collected in a lowland rainforest near Gua Musang, Malaysia, by C. Wiart in August 1995. The specimen of the plant (KL 4518) is deposited in the Herbarium of the Forest Research Institute of Malaysia at Kepory, Malaysia.

### 3.3. Extraction and isolation

Dried and powdered stem bark (1.5 kg) was macerated in 20% aq. MeOH (15 l) for 2 h and boiled for 3 h. The hydromethanolic extract was filtered, evaporated and freeze-dried to give a residue which was suspended in MeOH (120 ml). The methanolic fraction was added to 600 ml of $Me_2CO$ and the ppt. was filtered and dried over KOH *in vacuo* (2.5 g). The filtrate was evaporated, dried, suspended in MeOH and precipitated into $Et_2O$ and the ppt. was filtered and dried over KOH *in vacuo* (12.9 g). 2 g of the dried $Me_2CO$ ppt. was dissolved in pure $H_2O$ and dialysed against $H_2O$ in seamless cellulose tubing under agitation during 48 h. The contents of the tubes were freeze-dried to afford 1.6 g of a saponin mixt. A (yield 0.13%). An aliquot of the $Et_2O$ ppt. (7 g) was dialysed under agitation during 48 h and freeze dried to give 5.6 g of a saponin mixt. B (0.65%).

The saponin mixt. A (1.5 g) was fractionated on a silica gel VLC, using a gradient of $CHCl_3$/MeOH/$H_2O$ (14/6/1 to 12/8/1), to give 630 mg of purified saponin mixt., which was purified on a silicagel CC, using $CHCl_3$/MeOH/$H_2O$ (from 9/1/0 to 14/6/1). Frs. [15–26] eluted with $CHCl_3$/MeOH (85/15) were purified by prep. TLC in $CHCl_3$/MeOH/H2O (70/30/2) to give saponin **1** (2 mg). Frs. [43–50] (20.3 mg) eluted with $CHCl_3$/MeOH (85/15) were purified by semi-prep. HPLC with the gradient MeCN/$H_2O$ (from 55/45 to 58/42 during 30 min) to give compounds **4** (3 mg, rt = 12.9 min) and **5** (0.6 mg, rt = 11.7 min). Frs. [57–77] (27.4 mg) eluted with $CHCl_3$/MeOH (85/15) were purified by semi-prep. HPLC with the gradient MeOH/$H_2O$ (from 75/25 to 8/2 during 10 min and from 8/2 to 1/0 during 20 min) to give 2.6 mg of saponin **7** (rt = 22.7 min).

The saponin mixt. B (5.5 g) was fractionated on a silica gel VLC, using a gradient of $CHCl_3$/MeOH/$H_2O$ (from 14/6/1 to 12/8/1) to give 2 g of saponin extract, which was purified on a silicagel CC, using a gradient of $CHCl_3$/MeOH/H2O (from 9/1/0 to 12/8/1). Frs. [23–33] (235 mg) eluted with $CHCl_3$/MeOH (85/15) were purified on a reversed-phase RP-18 CC using MeOH/$H_2O$ (8/2). Frs. 7 (15.3 mg) and 10 (30.3 mg) were purified by preparative TLC in $CHCl_3$/MeOH/$H_2O$ (14/6/1) to give saponins **6** (1.6 mg), 2 (3.9 mg) and **5** (11.8 mg). Frs. [12–13] (20.6 mg) contained saponin **3** (3 mg) and Fr. [17] (6.8 mg) contained saponin **6** (1.4 mg), which were purified by semi-prep. HPLC with MeCN/$H_2O$ (4/6). Frs. [57–87] (221 mg) eluted with $CHCl_3$/MeOH (8/2) were purified on a silicagel CC with $CHCl_3$/MeOH (85/15) and then purified by semi-prep. HPLC with MeCN/$H_2O$ (6/4) to give 2.8 mg of saponin **8** (rt = 13.8 min).

### 3.4. Saponin 2

$[\alpha]_D^{21}$ + 4.92 (MeOH; *c* 0.32); $^1H$ and $^{13}C$ NMR ($CD_3OD$) of glycosidic part, see Table 1; $^{13}C$ NMR ($CD_3OD$) of aglycone δ 14.4 (C-25), 15.3 (C-24), 16.7 (C-26), 17.9 (C-6), 22.3 (C-16), 22.9 (C-30), 23.1 (C-11), 25.0 (C-27), 25.7 (C-2), 26.8 (C-23), 27.8 (C-15), 30.3 (C-20), 31.7 (C-22), 32.5 (C-29), 32.7 (C-7), 34.1 (C-21), 36.4 (C-10), 38.3 (C-1), 39.0 (C-4), 39.1 (C-8), 41.5 (C-14), 42.0 (C-18), 46.8 (C-17), 47.8 (C-19), 48.0 (C-9), 55.6 (C-5), 91.0 (C-3), 120.9 (C-12), 145.2 (C-13), 181.0 (C-28); ESI-MS (negative ion mode) *m/z* 911 $[M-H]^-$; ESI-MS-MS: $MS^2$ (911) *m/z* 779 $[M-H-132]^-$, 749 $[M-H-162]^-$, $MS^3$ (749) *m/z* 617 $[(M-H)-162-132]^-$, $MS^4$ (617) *m/z* 455 [genin-H]$^-$; ESI-MS (positive ion mode) *m/z* 935 $[M+Na]^+$; ESI-MS-MS: $MS^2$ (935) *m/z* 891 $[(M+Na)-CO_2]^+$, 803 $[(M+Na)-132]^+$, 773 $[(M+Na)-162]^+$, 497 [triglycosidic chain + Na]$^+$.

### 3.5. Saponin 3

$[\alpha]_D^{21}$ + 16.0 (MeOH; *c* 0.25); $^1H$ and $^{13}C$ NMR ($CD_3OD$) of glycosidic part, see Table 1; $^{13}C$ NMR ($CD_3OD$) of aglycone δ 14.4 (C-25), 15.4 (C-24), 16.3 (C-26), 17.8 (C-6), 22.5 (C-30), 22.6 (C-16), 23.1 (C-11), 24.9 (C-27), 25.6 (C-2), 26.8 (C-23), 27.4 (C-15), 30.2 (C-20), 32.1 (C-29), 32.4 (C-7), 32.5 (C-22), 33.5 (C-21), 36.4 (C-10), 38.3 (C-1), 39.0 (C-4), 39.1 (C-14), 41.3 (C-18), 41.4 (C-8), 45.8 (C-19), 46.3 (C-17), 47.7 (C-9), 55.5 (C-5), 90.8 (C-3), 122.1 (C-12), 143.8 (C-13), 180.7 (C-28); ESI-MS (negative ion mode) *m/z* 1043 $[M-H]^-$; ESI-MS-MS: $MS^2$ (1043) *m/z* 911 $[(M-H)-132]^-$, 881 $[(M-H)-162]^-$, 779 $[(M-H)-132\times2]^-$,749 $[(M^-H)-132-162]^-$, $MS^3$ (911) *m/z* 779 $[(M-H)-132\times2]^-$, 749 $[(M-H)-132-162]^-$, 455 [genin-H]$^-$; ESI-MS (positive ion mode) *m/z* 1067 $[M+Na]^+$; ESI-MS-MS: $MS^2$ (1067) *m/z* 1023 $[(M+Na)-CO_2]^+$, 935 $[(M+Na)-132]^+$, 905 $[(M+Na)-162]^+$, 803 $[(M+Na)-132\times2]^+$, 629 [tetraglycosidic chain + Na]$^+$.

### 3.6. Saponin 5

$[\alpha]_D^{21}$ + 10.5 (MeOH; *c* 0.98); $^1H$ and $^{13}C$ NMR ($CD_3OD$) of glycosidic part, see Table 1; $^{13}C$ NMR

($CD_3OD$) of aglycone $\delta$ 14.4 (C-25), 15.4 (C-24), 16.3 (C-26), 17.8 (C-6), 22.6 (C-30), 22.7 (C-16), 23.1 (C-11), 24.9 (C-27), 25.6 (C-2), 26.8 (C-23), 27.4 (C-15), 30.2 (C-20), 32.1 (C-29), 32.5 (C-7, C-22), 33.5 (C-21), 36.4 (C-10), 38.3 (C-1), 39.0 (C-4), 39.1 (C-8), 41.4 (C-14, C-18), 45.9 (C-19), 46.3 (C-17), 47.7 (C-9), 55.5 (C-5), 90.8 (C-3), 122.0 (C-12), 144 (C-13); ESI-MS (negative ion mode) $m/z$ 1073 $[M-H]^-$; ESI-MS-MS: $MS^2$ (1043) $m/z$ 941 $[(M-H)-132]^-$, 911 $[(M-H)-162]^-$, 779 $[(M-H)-132-162]^-$, $MS^3$ (779) $m/z$ 617 $[(M-H)-132-162\times2]^-$; ESI-MS (positive ion mode) $m/z$ 1097 $[M+Na]^+$; ESI-MS-MS: $MS^2$ (1097) $m/z$ 1053 $[(M+Na)-CO_2]^+$, 965 $[(M+Na)-132]^+$, 935 $[(M+Na)-162]^+$, 803 $[(M+Na)-132-162]^+$, 659 [tetraglycosidic chain + Na]$^+$.

### 3.7. Saponin 6

$[\alpha]_D^{21}$ −27.3 (MeOH; *c* 0.13); $^1H$ and $^{13}C$ NMR ($CD_3OD$) of glycosidic part, see Table 2; $^{13}C$ NMR ($CD_3OD$) of aglycone $\delta$ 14.4 (C-25), 15.3 (C-24), 16.2 (C-26), 17.8 (C-6), 22.5 (C-30), 22.6 (C-16), 23.1 (C-11), 24.9 (C-27), 25.7 (C-2), 26.8 (C-23), 27.4 (C-15), 30.1 (C-20), 32.1 (C-29), 32.3 (C-22), 32.5 (C-7), 33.4 (C-21), 36.4 (C-10), 38.3 (C-1), 39.1 (C-4, C-8), 41.3 (C-18), 41.4 (C-14), 45.7 (C-19), 46.2 (C-17), 47.5 (C-9), 55.5 (C-5), 90.4 (C-3), 122.2 (C-12), 143.7 (C-13), 180.3 (C-28); ESI-MS (negative ion mode) $m/z$ 881 $[M-H]^-$; ESI-MS-MS: $MS^2$ (881) $m/z$ 749 $[(M-H)-132]^-$, 719 $[(M-H)-162]^-$, 587 $[(M-H)-132-162]^-$; ESI-MS (positive ion mode) $m/z$ 905 $[M+Na]^+$; ESI-MS-MS: $MS^2$ (905) $m/z$ 861 $[(M+Na)-CO_2]^+$, 773 $[(M+Na)-132]^+$, 743 $[(M+Na)-162]^+$, 467 [triglycosidic chain + Na]$^+$.

### 3.8. Saponin 7

$[\alpha]_D^{21}$ +6.7 (MeOH; *c* 0.15); $^1H$ and $^{13}C$ NMR ($CD_3OD$) of glycosidic part, see Table 2; $^{13}C$ NMR ($CD_3OD$) of aglycone $\delta$ 14.4 (C-25), 15.3 (C-24), 16.4 (C-26), 17.0 (C-6), 22.6 (C-30), 24.4 (C-11), 24.9 (C-27), 26.2 (C-15), 26.7 (C-16, C-23), 27.5 (C-2), 30.6 (C-20), 32.2 (C-29), 32.5 (C-7), 33.2 (C-21), 33.6 (C-22), 36.4 (C-1), 38.3 (C-10), 39.1 (C-4, C-8), 41.4 (C-14), 41.5 (C-18), 46.5 (C-19), 48.3 (C-9), 55.5 (C-5), 90.6 (C-3), 121.6 (C-12), 144.2 (C-13); ESI-MS (negative ion mode) $m/z$ 911 $[M-H]^-$; ESI-MS-MS: $MS^2$ (911) $m/z$ 749 $[(M-H)-162]^-$, $MS^3$ (749) $m/z$ 587 $[(M-H)-162\times2]^-$, 455 [genin-H]$^-$; ESI-MS (positive ion mode) $m/z$ 935 $[M+Na]^+$; ESI-MS-MS: $MS^2$ (935) $m/z$ 891 $[(M+Na)-CO_2]^+$, 729 $[(M+Na)-CO_2-162]^+$.

### 3.9. Saponin 8

$[\alpha]_D^{21}$ +12.8 (MeOH; *c* 0.22); $^1H$ and $^{13}C$ NMR ($CD_3OD$) of glycosidic part, see Table 2; $^{13}C$ NMR ($CD_3OD$) of aglycone $\delta$ 14.4 (C-25), 15.3 (C-24), 16.5 (C-26), 17.9 (C-6), 22.7 (C-30), 23.1 (C-11), 24.9 (C-27), 25.7 (C-2), 26.0 (C-16), 26.8 (C-23), 27.6 (C-15), 30.3 (C-20), 32.3 (C-29), 32.6 (C-7, C-22), 33.8 (C-21), 36.4 (C-10), 38.3 (C-1), 39.0 (C-4), 39.1 (C-8), 41.5 (C-14), 41.6 (C-18), 46.3 (C-19), 48.2 (C-9), 55.5 (C-5), 90.5 (C-3), 121.5 (C-12), 144.4 (C-13), 177.5 (C-28); ESI-MS (negative ion mode) $m/z$ 1043 $[M-H]^-$; ESI-MS-MS: $MS^2$ (1043) $m/z$ 911 $[(M-H)-132]^-$, 881 $[(M-H)-162]^-$, 749 $[(M-H)-132-162]^-$, $MS^3$ (749) $m/z$ 587 $[(M-H)-132-162\times2]^-$, 455 [genin-H]$^-$; ESI-MS (positive ion mode) $m/z$ 1067 $[M+Na]^+$; ESI-MS-MS: $MS^2$ (1067) $m/z$ 1023 $[(M+Na)-CO_2]^+$, 935 $[(M+Na)-132]^+$, 905 $[(M+Na)-162]^+$, 773 $[(M+Na)-132-162]^+$, 629 [tetraglycosidic chain + Na]$^+$.

### 3.10. Acid hydrolysis of saponins

The crude saponin mixture B (500 mg) was refluxed with 30 ml of 2N HCl for 4 h 30. The sapogenin mixture was extracted with EtOAc (3×15 ml), washed with $H_2O$, dried over $Na_2SO_4$ and evapd. to dryness. Oleanolic acid and hederagenin were identified from the sapogenin residue with authentic samples by TLC in $CHCl_3$/MeOH (98/2). The acid aq. layer was neutralised with 0.5 M KOH and freeze-dried. Three sugars were identified with authentic samples by TLC in MeCOEt/*iso*/PrOH/$Me_2CO$/$H_2O$ (20/10/7/6) as glucose, galactose and arabinose. After prep. TLC of the sugar mixt. (100 mg) in this solvent, the optical rotation of each purified sugar was measured.

### 3.11. Haemolytic activity

This assay was performed as described previously (Voutquenne et al., 2002b). Sheep erythrocyte suspension (10%) was obtained by dilution of a commercial 50% suspension from Biomerieux® Lyon with phosphate buffer saline (PBS). Saponin mixt. was dissolved in PBS. 25 µl of erythrocyte suspension were added to 1 ml of the sample and rapidly stirred. Absorbance of the supernatant was measured at 540 nm after 60 min of incubation and centrifuged at 3000 rpm for 5 min. $HD_{50}$ and $HD_{100}$ were the concentrations of sample which cause 50 and 100% of haemolysis, respectively.

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