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Laurence Voutquenne*[,1], Catherine Lavaud[1], Georges Massiot[1,2], Louisette Le Men-Olivier[1].

[1] Laboratoire de Pharmacognosie, UMR CNRS 6013, Bât 18, BP 1039, 51097 Reims Cedex 2

[2] Present address, Institut de Recherche Pierre Fabre, CRSN, 3 rue Ariane, Parc Technologique du canal, 31527 Ramonville, France

* Laurence Voutquenne, UMR CNRS 6013, Bât 18, BP 1039, 51097 Reims Cedex 2

Fax number : 33 (0)3 26 91 35 96

E-mail : laurence.voutquenne@univ-reims.fr

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ABSTRACT

The haemolytic activity of 59 samples, natural or partially synthetic pure saponins and mixtures thereof was determined. The tested saponins possessed triterpenic genins and six structural skeleton types were studied. Structure-activity relationships were established by comparison of the functional groups of each saponin and of the branched sugar chains attached to aglycone. The haemolytic activity is shown to depend on the number of sugar units in the chains, as well as on the presence of an osidic chain in position 3 or of functionnal groups on the genin such as carboxylic or 16α-OH. Comparison of activities of monodesmosidic and bidesmosidic saponins showed that monodesmosidic saponins were generally more active and suggested a polar balance between the two sugar chains at positions 3 and 28. Twelve saponins showed strong haemolytic activity and five possessed a Haemolytic Index (HI) greater than 100000, such as the α and β aescines (HI : 200000).

KEY WORDS

Haemolytic activity, haemolytic Index, structure-activity relationships, triterpene saponins.

INTRODUCTION

The rupture of erythrocyte membranes (haemolysis) by saponins is one of the most spectacular properties of these molecules, having found application as reagents in blood cell counters (Hostettmann & Marston, 1995). The origin of the phenomenon is generally ascribed to their amphiphilic properties, but there is a lack of knowledge of the intimate molecular requirements for this activity. This fact is probably linked to the difficulties in obtaining sizeable quantities of pure material and in chemically modifying the structures. Recently, Oda et al. (2000) studied 47 triterpenic or steroidic saponins purified from medicinal and food plants to establish the relationship between adjuvant and haemolytic activities and concluded that there is no correlation. The purpose of this article is to report the investigation of the haemolytic activities of 59 compounds isolated from plants or produced by partial synthesis and try to deduce minimum structure activity relationships.

The 59 samples of this study are classified according to the structure of their genins (Fig. 1) and are glycosides of glycyrrhetinic acid (**g**), oleanolic acid (**o**), echinocystic acid (**e**), hederagenin (**h**), medicagenic acid (**m**), zanhic acid (**z**), protobassic acid (**pb**), 16α-hydroxy-protobassic acid (**hp**), protoprimulagenin A (**pp**), cyclamiretin A (**c**), protoaescigenin (**pa**), barringtogenol C (**b**), R1-barrigenol (**Rb**), A1-barrigenol (**Ab**), camelliagenin A (**cA**), asiatic acid (**a**), and soyasapogenols B and E (**sB** and **sE**).

(PLEASE INSERT FIGURE 1 HERE)

MATERIALS AND METHODS

Saponin samples

Tables 1 to 4 list the samples subjected to the study, arranged according to the nature of their triterpene. There are 53 pure compounds and 4 purified extracts of known composition characterized by a common genin. The panel was completed by the reference saponin compounds from Merck® and Sigma®, as well as commercially available chrysantellin A, α- and β-aescines, glycyrrhizin, asiaticoside, madecassoside and by partially synthetic compounds. The saponins were extracted from: leaves and roots of *Medicago sativa* (Fabaceae) (Massiot et al., 1988a,b, 1991), root bark of *Tridesmostemon claessenssi* (Sapotaceae) (Massiot et al., 1990), leaves of *Steganotaenia araliacea* (Apiaceae) (Lavaud et al., 1992), stem bark of *Petersianthus macrocarpus* (Lecythidaceae) (Massiot et al., 1992), leaves of *Aphloia madagascariensis* (Flacourtiaceae) (Dijoux et al., 1993), leaves and roots of *Beta vulgaris* (Chenopodiaceae) (Ridout et al., 1994 ; Massiot et al., 1994), stem bark of *Myrsine pellucida* (Myrsinaceae) (Lavaud et al., 1994a), root bark of *Smelophyllum capense* (Sapindaceae) (Lavaud et al., 1994b), leaves of *Pisonia umbellifera* (Nyctaginaceae) (Lavaud et al., 1996a), seed kernel of *Mimusops sp.* (Sapotaceae) (Lavaud et al., 1996b), stem bark of *Tapeinosperma clethroides* (Myrsinaceae) (Kitagawa et al., 1980; Lavaud et al., 1999), stem bark of *Filicium decipiens* (Sapindaceae) (Lavaud et al., 1998), stem bark of *Harpullia cupanioides* (Sapindaceae) (Dimbi et al., 1983; Voutquenne et al., 1998), and leaves of *Desmodium adscendens* (Fabaceae) (McManus et al., 1993).

The oleanolic derivatives **o-1** to **o-4** were prepared from oleanolic acid (**o**) and the corresponding peracetobromosugars under phase transfer conditions (Bliard et al., 1994). The same conditions were used for the preparation of **m-5** and **m-6** from **m-3**, of **a-1** from asiatic acid (**a**) and of **g-2** from glycyrrhetinic acid (**g**). Saponin **o-5** was prepared from **o-1** by Koenigs-Knorr condensation with acetyl lactosyl bromide (Nazabadioko, 1996). Partial acid hydrolysis of α-hederin (**h-2**) in 0.04 N H_2SO_4 in 50% EtOH solution provided **h-1**. Partial alkaline hydrolysis in 1% KOH in 30% EtOH solution of bidesmosidic saponins **e-2**, **h-6** or

ester saponins **Rb1**, **pa-1** and **pa-2** gave the corresponding monodesmosidic or saponified saponins **e-1**, **h-7**, **Rb-4**, and **pa-3**.

The commercially saponins Merck®, mixture from *Gypsophyla paniculata* (Caryophyllaceae), and Sigma®, mixture from *Quillaja saponaria* (Rosaceae), were dialysed in Spectra/Por® molecular porous membrane tubing with MCWO : 6- 8000. Saponins (1 g) were dissolved in 15 ml of pure water and the solution was introduced into the Spectra/Por® membrane tubing. The saponin mixture was dialysed against pure water for 72 h and then the content of the tube was lyophilised. The powdered residues constituted the reference saponin mixtures Merck®D and Sigma®D (175 mg, yield 17.5%), respectively.

(PLEASE INSERT TABLES 1 TO 4 HERE)

Haemolysis test

Material: : Sheep erythrocytes were purchased from Biomerieux-Lyon as a 50% suspension and diluted with phosphate buffer saline (PBS) to give a 10% suspension. Saponins were dissolved in PBS and pH was adjusted to 7.4; 2 ml samples were prepared with concentrations ranging from 10^{-3} mg/ml to 1 mg/ml. Each sample was tested at least twice. In the case of sapogenins, the mother solution was a mixture of DMSO : water (5 : 1); further dilutions were made with PBS (Segal et al., 1974).

Test : The erythrocyte suspension (50 µl) was added to 2 ml of the sample to be tested and the mixture was rapidly stirred, incubated for 60 min at room temperature, then centrifuged at 3000 rpm for 5 min. Absorbance of the supernatant was measured at 540 nm. The 100% haemolytic dose (HD_{100}) was determined as the saponin concentration (µg/ml or µM) inducing the same haemolysis that was caused by Sigma® dialyzed mixture (Sigma® D). For this sample, 100% haemolysis (HD_{100}) was obtained at a concentration of 75 µg/ml. The haemolytic index (HI) was calculated according to the European Pharmacopoeia as HI= 30

000 x (a) / (b) where (a) and (b) are the concentrations in (g/l) of the saponin reference "Merck® D" and of the saponin under study which gives 100% haemolysis.

Haemolytic time course measurements

The erythrocyte suspension (50 µl) was added to 2 ml of commercial saponins whose final concentrations caused 100% haemolysis within 60 min, and the mixture was rapidly stirred, incubated at room temperature. After 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 15, 30 or 60 min of incubation, the whole mixture was centrifuged at 3000 rpm for 5 min. The absorbance of each supernatant was measured at 540 nm. The value % haemolysis was the ratio of this absorbance to that obtained at 100% haemolysis within 60 min.

RESULTS

The commercial saponins from Merck® and Sigma® possessed a 100% haemolytic dose (HD_{100}) at a concentration of 250 µg/ml. A similar activity is obtained at respective concentrations of 50 and 75 µg/ml after a step of purification of these mixtures by dialysis against pure water. Fig. 2 shows representative examples of haemolysis curves in which absorbance is plotted against concentration. The series of data are obtained with saponins **c-1** to **c-3**, **pp-1** and **h-4** containing cyclamiretin A (**c**), protoprimulagenin A (**pp**) and hederagenin (**h**) as aglycone and the dialysed reference saponin from Sigma®. All the curves are of the sigmoidal type which means that the haemolysis needs a minimum concentration to start, after which the reaction rapidly takes place.

(PLEASE INSERT FIGURE 2 HERE)

Fig. 3 compares the haemolytic time courses of the two dialysed reference saponin from Merck® and Sigma® with three commercially available saponins α-hederin (**h-2**),

chrysantellin A (**e-2**) and α-aescine (**pa-1**). The time courses of the reference compounds are similar and 90% haemolysis is obtained in one minute. The haemolytic rates of the pure commercial saponins **h-2** and **e-2** are fast (15 sec) while that of the mixture **pa-1** is slow (10 min) compared to the reference saponins. The difference in the time course is not easily explained but it seems that the haemolytic time course of pure saponins is faster than the mixture. Takechi and Tanaka (1995) have previously mentioned that differences in haemolytic time courses could be due to structural differences of the sapogenins and not of the sugars moieties as proposed by Segal et al. (1974).

(PLEASE INSERT FIGURE 3 HERE)

The results presented in Table 1 show that monodesmosidic saponins with an ester chain at C-28 (**o-1** to **o-4**) or C-30 (**g-2**) are not haemolytic. The same result is observed with the monodesmosidic ursane derivatives: **a-1** to **a-3** and with saponins from *Aphloia madagascariensis* (Table 4). The introduction of an osidic residue in position 3 to the saponin **o-1** gives the bidesmosidic saponin **o-5** which displays a low haemolytic activity.

The comparison of haemolytic potency of three saponins monoglycosylated in position 3 with sapogenins **h-1**, **e-1** and **m-1**, shows that the presence of one additional hydroxyl group at position 2 (**m-1**) or 16α (**e-1**) multiplies the activity by a factor of fifteen (Fig. 4). A similar effect is observed between the bidesmosidic saponins **hp-1** and **hp-2** which contain an hydroxyl group at position 16α and saponins **pb-1** and **pb-2** which are about three times less active (Table 1).

(PLEASE INSERT FIGURE 4 HERE)

The haemolytic activity of monodesmosidic saponins containing a 13β,28-epoxygenin as cyclamiretin A (**c**) (Table 2) and of similar monodesmosidic saponins with hederagenin (**h**) (Table 1) were compared. The bi- or triglycosylated saponins **c-2** and **c-3** exhibit the same range of haemolytic activity as **h-2**, **h-3**, **h-4** and **h-7**, suggesting that the genins induce

similar effects. When the ether ring is cleaved into a carbinol at position C-28 (**c-4**), the haemolytic activity is strongly reduced. Comparison of activity of **c-4** with that of **e-1** shows that the carboxylic function at position C-28 enhances the activity.

Table 3 reports the haemolytic activity of saponins with a polyhydroxylated sapogenin in rings D or E. The α and β aescines **pa-1** and **pa-2** which contain two free hydroxyls and two esters in ring D and/or E are strongly haemolytic with HD_{100} 6.6 μM as observed by Oda et al. (2000). The saponins extracted from *Harpullia cupanioides* show a strong activity and their structures possess two esters and two free hydroxyls. A decrease in activity is observed with saponins containing less than two ester functions like saponins **b-1** and **b-2** from *Petersianthus macrocarpus*. No activity appears with saponins which contain four or more free hydroxyls such as the *Steganotaenia* saponins (**Rb-1** to **Rb-3**) and the products **pa-3** and **Rb-4** resulting from alkaline hydrolysis. Monodesmosidic diesterified saponins with protoaescigenin (**pa**) and barringtogenol (**b**) (Table 3) possess the same activity or are as much as ten-fold more active than the corresponding monodesmosidic saponins with cyclamiretin A (**c**) (Table 2), hederagenin (**h**) or medicagenic acid (**m**) (Table 1) as aglycones.

The saponin extract from *Desmodium adscendens* containing soyasaponin I (**sB1**), III (**sB-2**) and dehydrosoyasaponin I (**sE-1**) whith one hydroxyl in ring E is inactive (Table 1). This seems to be due to the free hydroxyl in position 22 and to the absence of a carboxylic group in position 28. This absence of haemolytic activity of soyasaponins has been previously related by Gestetner et al. (1971) and more recently by Oda et al. (2000). Thus, the inactivity of the glycyrrhizin (**g-1**) can be explained by the absence of carboxylic group at C-28 (Table 1).

From the results presented in Table 1, it appears that the haemolytic activity of di-and triglycosides saponins **h-2**, **h-3**, **h-4** and **h-7** is higher than that of the monoglycoside **h-1**, so the number of sugars residues also affects the haemolytic activity of monodesmosidic saponins. The saponins **c-1** and **pp-1** with four or five osidic units are more active than

triglycosidic saponins **c-2** and **c-3** (Table 2, Fig. 3). The activity of monodesmosidic saponin in C-3 increases with the number of sugar units and is maximum with four sugar units. The decrease of activity between **c-1** and **pp-1** can be due to the increased number of osidic units. Abe et al. (1978) has previously reported that haemolytic activity of saikosaponins depends on the number of sugars.

In Fig. 5, haemolytic potency of monodesmosidic saponins **m-1**, **h-2** and **h-7** is compared with one of the corresponding bidesmosidic saponins **m-2**, **h-5** and **h-6**. It shows that glucosylation in C-28 decreased the activity. The monodesmosidic medicoside A (**m-1**) shows the greatest activity compared to the bidesmosidic medicosides G and J (**m-2** and **m-3**) and medicoside **m-4** as previously reported by Oleszek (1990) with alfalfa saponins (Table 1).

(PLEASE INSERT FIGURE 5 HERE)

Comparison of the haemolytic activity of bidesmosidic saponins with only one glucose unit in position 28 like **o-5**, **o-6**, **h-5**, **h-6**, **m-2,** and saponin extracts of *Pisonia umbellifera* and of *Beta vulgaris*, shows activity increasing with the number of sugars branched in position 3 as previously observed for monodesmosidic saponins and with a maximum for three sugars units as in saponin **h-6** (Table 1). In bidesmosidic saponins with the same trisaccharide in position 28 of medicagenic acid, haemolytic activity is related to the number of sugars attached to the saponin, **m-3** being the most active. Increasing the number of sugar branches on ring A at positions 3 or 23 (saponins **m-4**, **m-5** and **m-6**) has a negative effect on activity (Table 1).

In the case of saponins with one tetrasaccharidic ester chain as saponins **pb-1**, **pb-2** and **pb-3**, the highest activity is observed for **pb-3** with two glucoses in the chain at C-3 (Table 1). A similar effect is observed for the couple of saponins **m-7** and **m-4**. A strong activity is observed in saponin **z-1** with the same ester chain but only with one glucose at C-3, this may result from the beneficial effect of the 16α-hydroxyl to the haemolytic activity (Table 1). One

supplementary sugar in the ester chain at position 28 as in saponins **hp-3** and **hp-4** decreases activity. The activity is best when only one sugar constitutes the chain at C-3 (Table 1).

DISCUSSION

Table 5 gives a classification of the saponins according to their haemolytic index (HI) with comparison with a commercial saponin Merck®. We distinguish saponins with low (HI<10 000), moderate (10 000 ≤ HI ≥ 30 000) and strong haemolytic activity (HI>50 000). The table also includes some important structural features of the molecules. Results of this investigation lead to the conclusion that monodesmosidic and bidesmosidic saponins must be separated, for establishment of structure-activity relationships. Comparison of the haemolytic index shows that monodesmosidic saponins are generally more active than bidesmosidic saponins.

(PLEASE INSERT TABLE 5 HERE)

Monodesmosidic saponins with a single ester chain at position 28 are not haemolytic. The introduction of an osidic residue in position 3 gives haemolytic potency thus confirming the work of Hase et al. (1981). The haemolytic potency of monoglycosidic saponins in position 3 increases with the presence of one α-hydroxyl at position 16 or at position 2 as previously described by Schlösser and Wulff (1969) which noticed that for activity to be observed, the aglycone requires a polar group in ring A (OH and/or COOH) and a moderately polar group in ring D or E. Saponins with a 13β,28-epoxy bridge are highly haemolytic and this activity is reduced when the ether ring is cleaved (Nose et al., 1989; Sindambiwe et al., 1998). Saponins without a carboxylic group in C-28 are inactive as with the soyasaponins (Gestetner et al., 1971; Oda et al., 2000). These results allow to conclude that haemolytic activity of monodesmosidic saponins in position 3 was connected with a carboxylic function

at position 28 or a 13β,28-epoxy bridge, and with one α-hydroxyl at position 16. This activity increases with an alcohol function in ring A at positions 2 or 23 or in ring B at position 6.

The presence of many free hydroxyls in the ring E as in the saponins **Rb-1** to **Rb-4** and **pa-3**, induces loss of haemolytic activity as previously announced by Hase et al. (1981) and Schlösser and Wulff (1969). The esterification of these hydroxyls gives haemolytic potency as in saponins from *Harpullia cupanioides* and the aescines (Table 3). These results confirm the previous reported findings of Segal et al. (1966, 1970) on activity enhancement by esterification, independant of the type of ester, as well as of Schlösser and Wulff (1969) on the requirement for the genin to possess a limited number of OH groups in rings D and/or E to be active. In our case, the presence of one ester at position 22 seems to be necessary to display haemolytic activity as in the aescines **pa-1** and **pa-2** (Table 5). We confirm that polarity in ring A and relative hydrophobicity in rings D/E are essential for haemolytic activity (Hostettmann & Marston, 1995).

Moreover we observe with the two types of genins (hederagenin and cyclamiretin A) that the haemolytic activity of monodesmosidic saponins increases with the number of sugar units as Romussi et al. (1980) and Takechi and Tanaka (1992) have observed with oleanolic acid saponins, and Abe et al. (1978) with saikosaponins. Haemolytic activity of monodesmosidic saponins in position 3 is thus higher with three or four sugars units in the sugar chain.

Comparison of monodesmosidic saponins and corresponding bidesmosidic saponins shows that glucosylation in C-28 decreases the haemolytic activity as previously reported by Hase et al. (1981). In the case of bidesmosidic saponins, with one ester sugar unit at the position 28, the presence of three sugars at the position 3 is necessary to keep a moderate activity. Whereas when the number of sugars in the sugar chain at position 28 is higher than four or five units, the number of sugars in the sugar chain at position 3 must be decreased to one sugar. These results suggest the necessity of a polar balance between the two sugar chains at positions 3 and 28. The activity of bidesmosidic saponins is the highest when the osidic

ester chain in position 28 is ramified with four sugars and one glycoside in position 3 (Table 5). As in monodesmosidic saponins, the presence of a 16α-OH increases the haemolytic activity. One hydroxyl in position 2 and of a polar function like COOH or CH_2OH in C-23 seem to be necessary for haemolytic activity.

All these results do not include the nature of the sugar unit in structure-activity relationships. The presence of one terminal xylose in saponin **h-3** slightly decreases the activity as compared to the arabinose in saponin **h-4**. The glucose unit in the middle of the chain of saponin **h-7** decreases the activity in comparing to the presence of rhamnose as in saponin **h-4** (Table 1). The presence of glucuronic acid seems to decrease activity as suggested by the comparison of saponins **pb-1**, **hp-1** and **o-5** which contain one glucose linked to oxygen-3 with the saponins **pb-2**, **hp-2** and **o-6** where one glucuronic acid takes the place of glucose.

Twelve saponins are found more active than the saponin mixture Merck® D which is known to possess a HI of 30 000 (Table 5). They are the bidesmosidic saponins **hp-1**, **pb-3** and tridesmosaponin A (**hp-4**) isolated from *Mimusops sp.*, the saponin **m-7** isolated from *Filicium decipiens* and the chrysantellin A (**e-2**). The monodesmosidic active saponins are α-hederin (**h-2**), sapindoside B (**h-3**) and sapindoside **h-4**, the saxifragoline B (**c-1**), the sakuraso-saponin (**pp-1**), the α-and β-aescines (**pa-1**, **pa-2**).

In this study, we have attempted to increase the knowledge of haemolytic activity of triterpene saponins. The great variability in the structure of saponins makes this type of study delicate and the results may be difficult to rationalize. New structure-activity relating to the chemical structure of the aglycon and of the sugar chains have been proposed in this work. In order further to confirm the above assumptions as well as investigate how the nature and the sequencing osidic units affect haemolytic activity, it is necessary to test many other saponins from various sources. This study should be completed with haemolytic tests on human blood cells.

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Table 1. Haemolytic activity of saponins with carboxylic function in sapogenin part.

	OR-3	COOR-23, 28 or 30	Trivial name	HD_{100} μ*M* (μg/mL)	reference
o	H	H- 28	oleanolic acid	> 2200	-
o-1	H	Glc- 28	-	> 1600 [15%]	Bliard, 1994
o-2	H	Glc-^{4}Glc- 28	-	nha	Bliard, 1994
o-3	H	Rha-^{6}Glc-^{6}Glc- 28	-	nha	Bliard, 1994
o-4	H	Glc^{6}Gal-^{4}Glc– 28 (Glc at 6)	-	> 900 [15%]	Bliard, 1994
o-5	Gal-^{4}Glc-	Glc- 28	calenduloside B	1061 [96%] (1)	-
o-6	Gal-^{2}GlcA-	Glc- 28	-	> 523 [0%]	Lavaud, 1992
e-1	Glc-	H- 28	-	118 (75)	-
e-2	Glc-	Rha-^{3}Xyl-^{4}Rha-^{2}Xyl- 28	chrysantellin A	16.8 (20)	-
***Pisonia* extract**	1, 2 or 4 sugars	Glc- 28		≈ 75 μg/ml	Lavaud, 1996a
***Beta l.* extract**	3 sugars	H or Glc- 28		≈ 500 μg/ml	Ridout, 1994 Massiot, 1994
h	H	H-28	hederagenin	> 2000 [68%]	-
h-1	Ara-	H-28	fatsiaside B1	1656 (1000)	-
h-2	Rha-^{2}Ara-	H-28	α-hederin	26.7 (20)	Lavaud, 1994b
h-3	Xyl-^{3}Rha-^{2}Ara-	H-28	sapindoside B	22.7 (20)	Lavaud, 1994b
h-4	Ara-^{3}Rha-^{2}Ara-	H-28	-	11.3 (10)	Lavaud, 1994b
h-5	Ara-^{2}Rha-	Glc- 28	-	1096 (1000)	Lavaud, 1994b
h-6	Ara-^{2}Glc-^{2}Ara-	Glc- 28	medicoside I	94.3 (100)	Massiot, 1988, 1991
h-7	Ara-^{2}Glc-^{2}Ara-	H- 28	medicoside C	55.7 (50)	-
pb-1	Glc-	Rha-^{3}Xyl-^{4}Rha-^{2}Ara- 28	Mi-saponin A	61.4 (75)	Lavaud, 1996b
pb-2	GlcA-	Rha-^{3}Xyl-^{4}Rha-^{2}Ara- 28	butyroside C	202 (250)	Lavaud, 1996b
pb-3	Glc-^{3}Glc-	-Rha-^{3}Xyl-^{4}Rha-^{2}Ara- 28		18.1 (25)	Lavaud, 1996b
hp-1	Glc-	Rha-^{3}Xyl-^{4}Rha-^{2}Ara- 28	arganine C	20.2 (25)	Lavaud, 1996b
hp-2	GlcA-	Rha-^{3}Xyl-^{4}Rha-^{2}Ara- 28		59.9 (75)	Lavaud, 1996b
hp-3	Glc-^{3}Glc-	Rha-^{3}Xyl–^{4}Rha–^{2}Xyl– 28 (Rha at 3)	tridesmosaponin B	162 (250)	Massiot, 1990

hp-4	Rha-	Rha-^{3}Xyl(^{4}Rha)-^{4}Rha-^{2}Xyl- 28	tridesmosaponin A	54.8 (75)	Massiot, 1990
m-1	Glc-	H- 28, H- 23	medicoside A	90.4 (60)	Massiot, 1988
m-2	Glc-	Glc- 28, H- 23	medicoside G	> 605 [0%]	1991
m-3	Glc-	Xyl-^{4}Rha-^{2}Ara- 28, H- 23	medicoside J	233 (250)	Massiot, 1988
m-4	Glc-^{2}Glc-	Xyl-^{4}Rha-^{2}Ara- 28, H- 23		809 (1000)	1991
m-5	Glc-	Xyl-^{4}Rha-^{2}Ara- 28, Glc- 23		405 (500)	-
m-6	Glc-	Xyl-^{4}Rha-^{2}Ara- 28, Gal-^{4}Glc- 23		715 [82%] (1000)	-
m-7	Glc-^{2}Glc-	Xyl-^{4}Rha-^{2}Fuc- 28, H-23 (^{2}Ara, ^{4}Nil-Nil)		15.8 (25)	Lavaud, 1998
z-1	Glc-	Xyl-^{4}Rha-^{2}Fuc- 28, H-23 (^{2}Ara, ^{4}Nil-Nil)		34.8 (50)	Lavaud, 1998
sB-1	Rha-^{2}Gal-^{2}GlcA-	-	soyasaponin I	nha	Mc Manus,
sB-2	Gal-^{2}GlcA-	-	soyasaponin III	nha	1993
sE-1	Rha-^{2}Gal-^{2}GlcA-	-	dehydrosoyasaponin I	nha	"
g-1	(GlcAO-NH_4^+)$_2$-H	NH_4^+- 30	glycyrrhizin	nha	-
g-2		Glc-^{4}Glc- 30		nha	-

nha : no haemolytic activity. Each monosaccharide unit is abbreviated as follows : arabinose (Ara), xylose (Xyl), glucose (Glc), glucuronic acid (GlcA), galactose (Gal), fucose (Fuc), rhamnose (Rha). Acyls groups are described as follows : acetyl (Ac), niloyl (Nil), angeloyl (Ang) and tigloyl (Tig).

Table 2. Haemolytic activity of cyclamiretin A (**c**) and protoprimulagenin A (**pp**).

	OR-3	C-28	Trivial name	HD_{100} µM (µg/mL)	reference
c-1	Xyl—^{2}Glc—^{4}Ara(^{2}Glc)—	13,28-epoxy	saxifragoline B	7.1 (7.5)	Lavaud, 1994a
c-2	Xyl-^{2}Glc-^{4}Ara-	13,28-epoxy	primulanin	55.7 (50)	Lavaud, 1994a
c-3	Rha-^{2}Glc-^{4}Ara-	13,28-epoxy	-	54.8 (50)	Lavaud, 1994a
c-4	Xyl—^{2}Glc—^{4}Ara(^{2}Glc)—	CH_2OH	-	> 472 [0%]	Lavaud, 1994a
pp-1	Rha—^{2}Rha—^{2}Gal—^{3}GlcA(^{2}Glc)	13,28-epoxy	sakuraso-saponin	12 (15)	Lavaud, 1999

Table 3. Haemolytic activity of saponins with a polyhydroxylated ring D or E in sapogenin part.

	OR-3	OR-21	OR-22	OR-28	Trivial name	HD100 μM (μg/mL)	reference
pa-1	Glc—^{4}GlcA— 2| Glc	Ang-	CH_3CO-	H	α-aescine	6.6 (7.5)	-
pa-2	Glc—^{4}GlcA— 2| Glc	Tig-	CH_3CO-	H	β-aescine	6.6 (7.5)	-
pa-3	Glc—^{4}GlcA— 2| Glc	H	H	H	-	> 497 [0%]	-
b-1 + **b-2**	Gal—^{3}GlcA— 2| Gal	Tig-Nil-Ara- Tig Ph-CO-	H H	H Rha-	- -	76.2 (100)	Massiot, 1992
Rb-1	Gal—^{3}GlcA— 2| Gal	Tig-	H	H		> 460 [0%]	Lavaud, 1992
Rb-2	Gal—^{3}GlcA— 2| Gal	Ang-	H	H		> 460 [0%]	Lavaud, 1992
Rb-3	Xyl—^{4}GlcA— 2| Glc	Ang-	H	H		> 473 [0%]	Lavaud, 1992
Rb-4	Gal—^{3}GlcA— 2| Gal	H	H	H	-	> 497 [0%]	–
***Harpullia* extract**	Rha—^{3}GlcA— 2| hexose	-	H or Ang	Ac or Ang	-	9.6	Voutquenne, 1998

Table 4. Haemolytic activity of saponins derivatives of ursane skeleton.

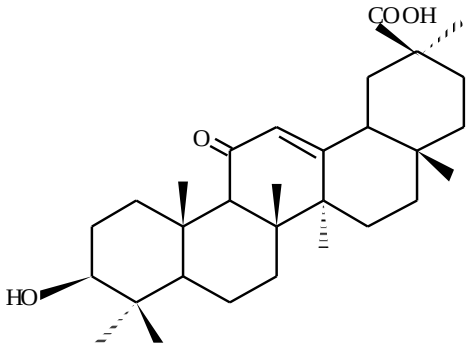
	R^1-23	**R^2-6**	**R^3-19**	**COOR-28**	**Trivial name**	**HD_{100} µM**	**reference**
a-1	OH	H	H	Glc-^{4}Glc-	-	nha	-
a-2	OH	H	H	Rha-^{4}Glc-^{6}Glc-	asiaticoside	nha	-
a-3	OH	OH	H	Rha-^{4}Glc-^{6}Glc-	madecassoside	nha	-
***Aphloia* extract**	H or OH	OH	OH	Glc-	-	nha	Dijoux, 1993

nha : no haemolytic activity

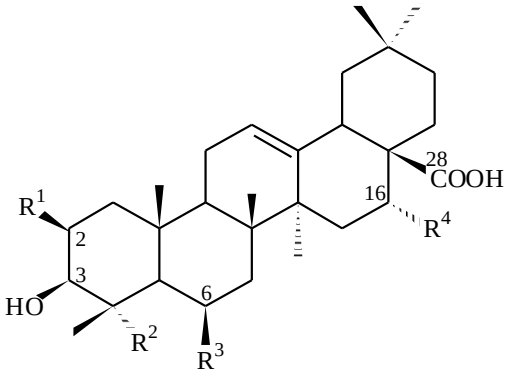
Table 5. Haemolytic index of tested saponins.

Saponin	HI	ether ring 13-28 or COOH-28	OH-16 α	Ester	Number of sugars in C-3/C-28
h-1	1 500	+	-	-	1/0
m-4	1 500	-	-	-	2/3
m-3	6 000	-	-	-	1/3
hp-3	6 000	-	+	-	2/3
h-6	15 000	-	-	-	3/1
b-1+b-2	15 000	-	+	+	3/1
pb-1	20 000	-	-	-	1/4
Sigma® D	**20 000**	**-**	**+**	**++**	**3/≥5**
z-1	30 000	-	+	+	1/4
e-1	20 000	+	+	-	1/0
c-2	30 000	+	+	-	3/0
c-3	30 000	+	+	-	3/0
h-7	30 000	+	-	-	3/0
Merck® D	**30 000**	**-**	**+**	**++**	**3/≥5**
m-7	60 000	-	-	+	2/4
pb-3	60 000	-	-	-	2/4
hp-1	60 000	-	+	-	1/4
hp-4	60 000	-	+	-	1/5
e-2	75 000	-	+	-	1/4
h-2	75 000	+	-	-	2/0
h-3	75 000	+	-	-	3/0
pp-1	100 000	+	+	-	5/0
h-4	150 000	+	-	-	3/0
Harpullia	150 000	-	+	++	3/0
c-1	200 000	+	+	-	4/0
pa-1	200 000	-	+	++	3/0
pa-2	200 000	-	+	++	3/0

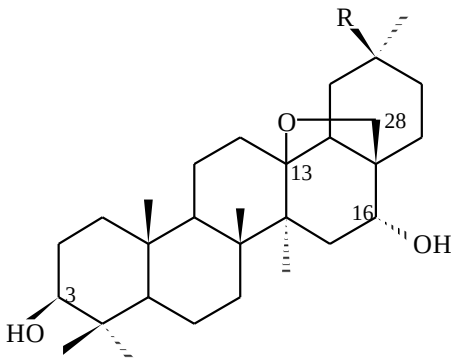
++ two or more ester functions



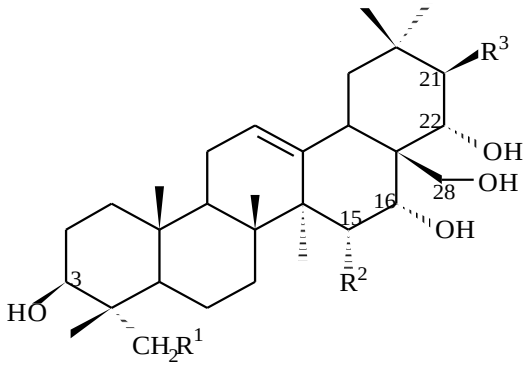
g glycyrrhetinic acid



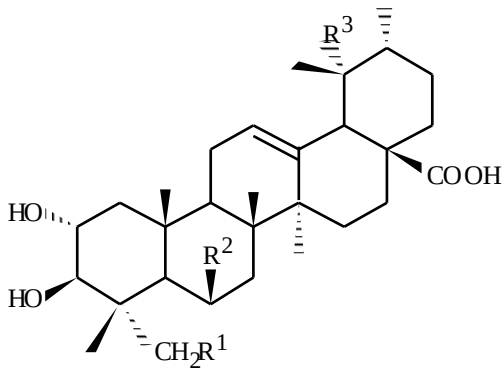
		R^1	R^2	R^3	R^4
o	oleanolic acid	H	CH_3	H	H
e	echinocystic acid	H	CH_3	H	OH
h	hederagenin	H	CH_2OH	H	H
m	medicagenic acid	OH	COOH	H	H
z	zanhic acid	OH	COOH	H	OH
pb	protobassic acid	OH	CH_2OH	OH	H
hp	16α-hydroxy-protobassic acid	OH	CH_2OH	OH	OH



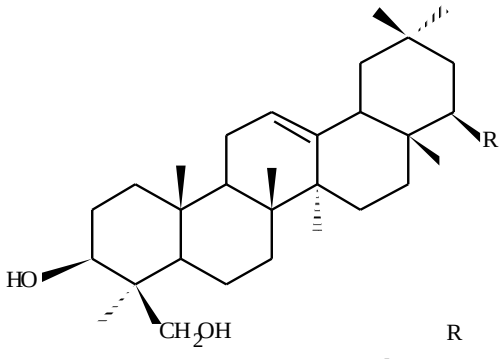
		R
pp	Protoprimulagenin A	CH_3
c	cyclamiretin A	CHO



		R^1	R^2	R^3
pa	protoaescigenin	OH	H	OH
b	barringtogenol C	H	H	OH
Rb	R1-barrigenol	H	OH	OH
Ab	A1-barrigenol	H	OH	H
cA	camelliagenin A	H	H	H



a asiatic acid R^1=OH, R^2= R^3= H



		R
sB	soyasapogenol B	OH
sE	soyasapogenol E	=O

Fig. 1. Structures of genins of tested saponins.

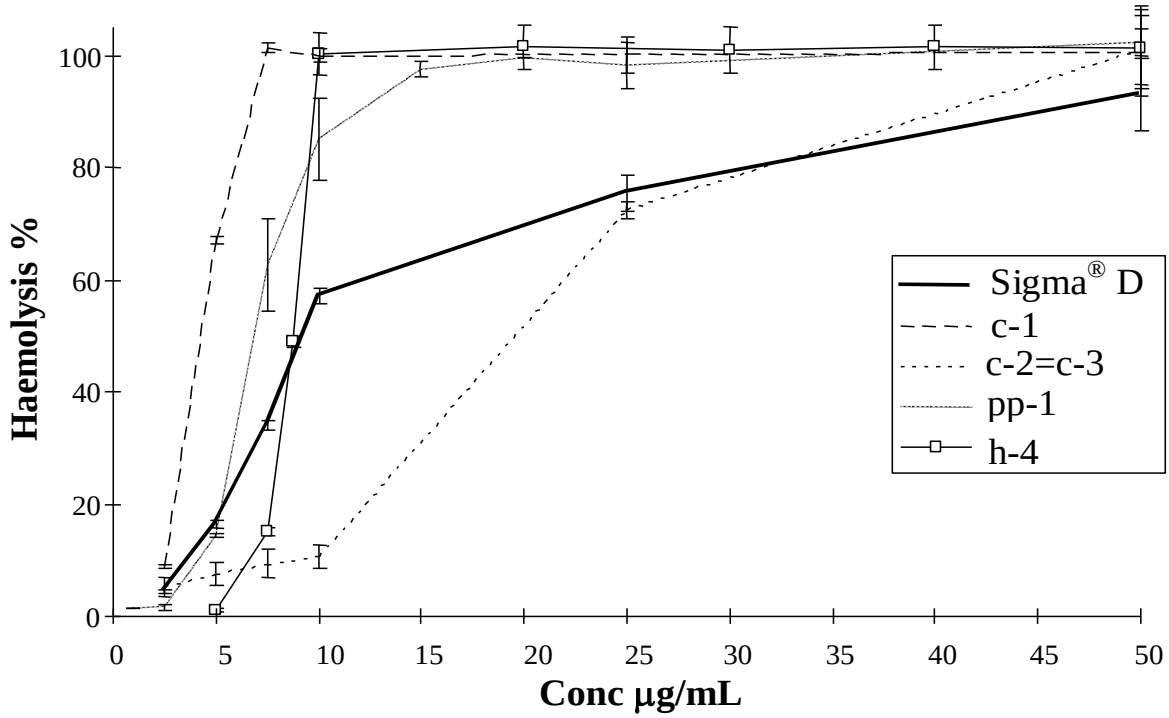


Fig. 2. Haemolytic activity of saponins with cyclamiretin A (**c**), protoprimulagenin A (**pp**) and hederagenin (**h**).

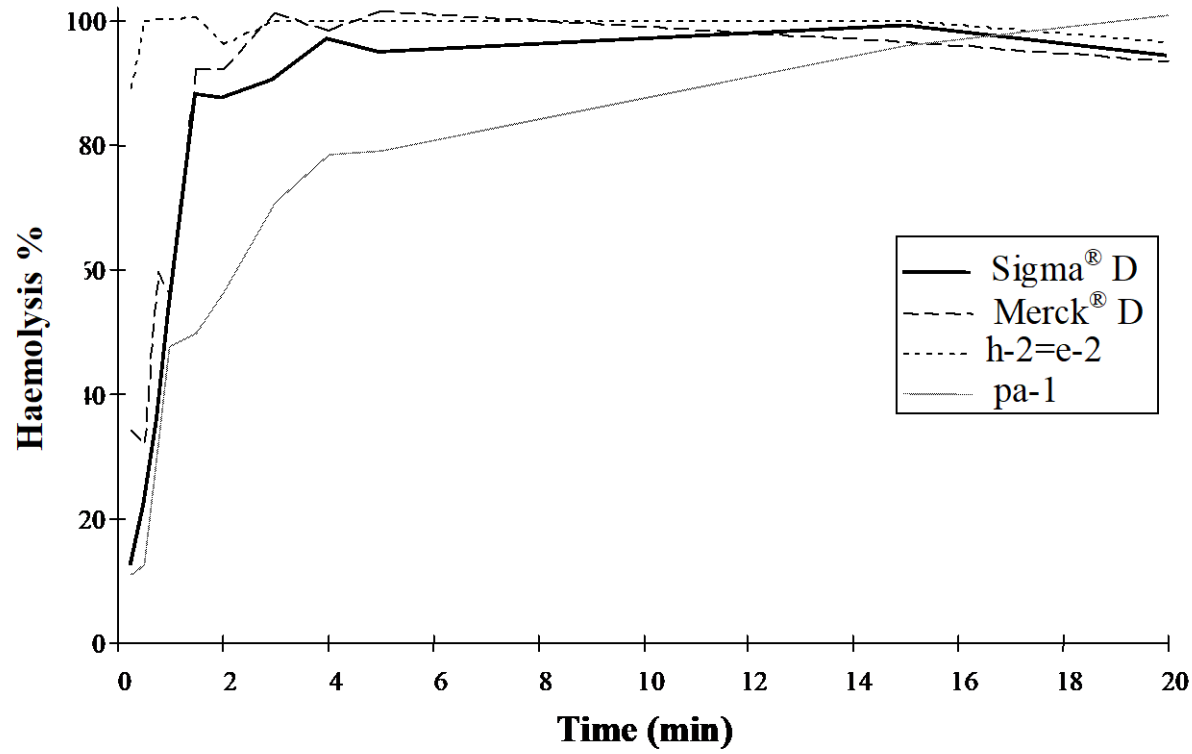


Fig. 3. Haemolytic time course measurements.

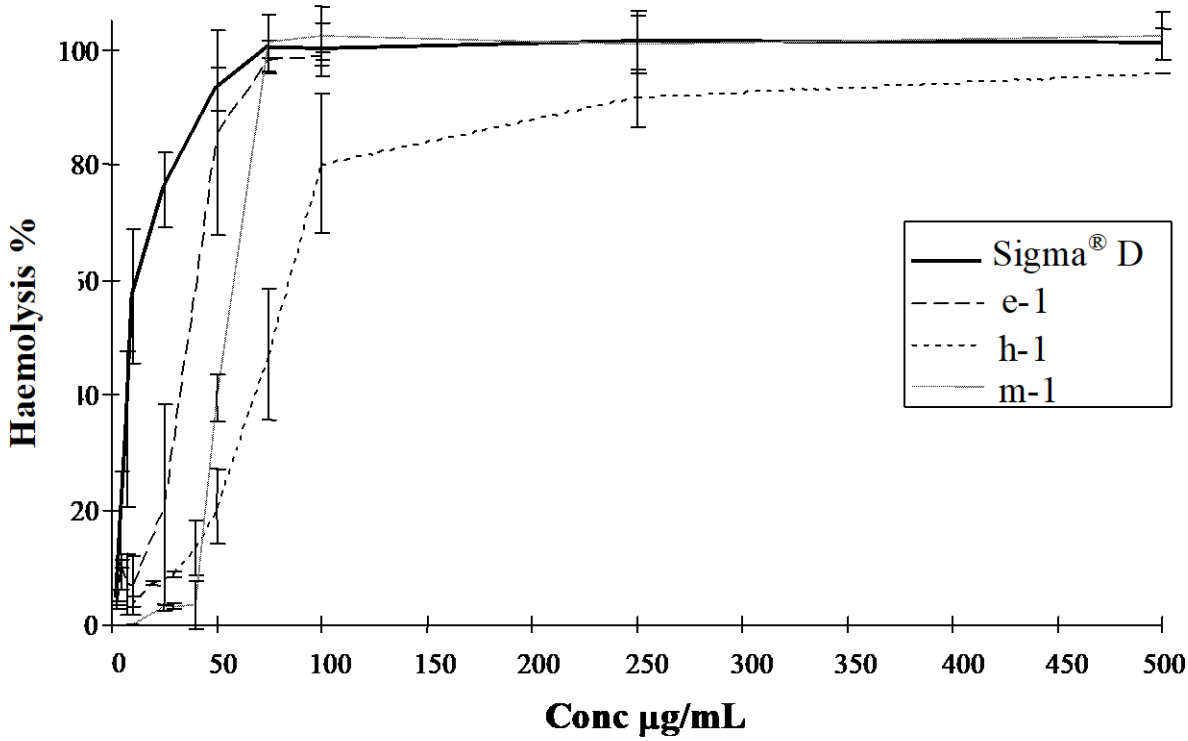


Fig. 4. Influence of one polar group in rings A or D on haemolytic activity.

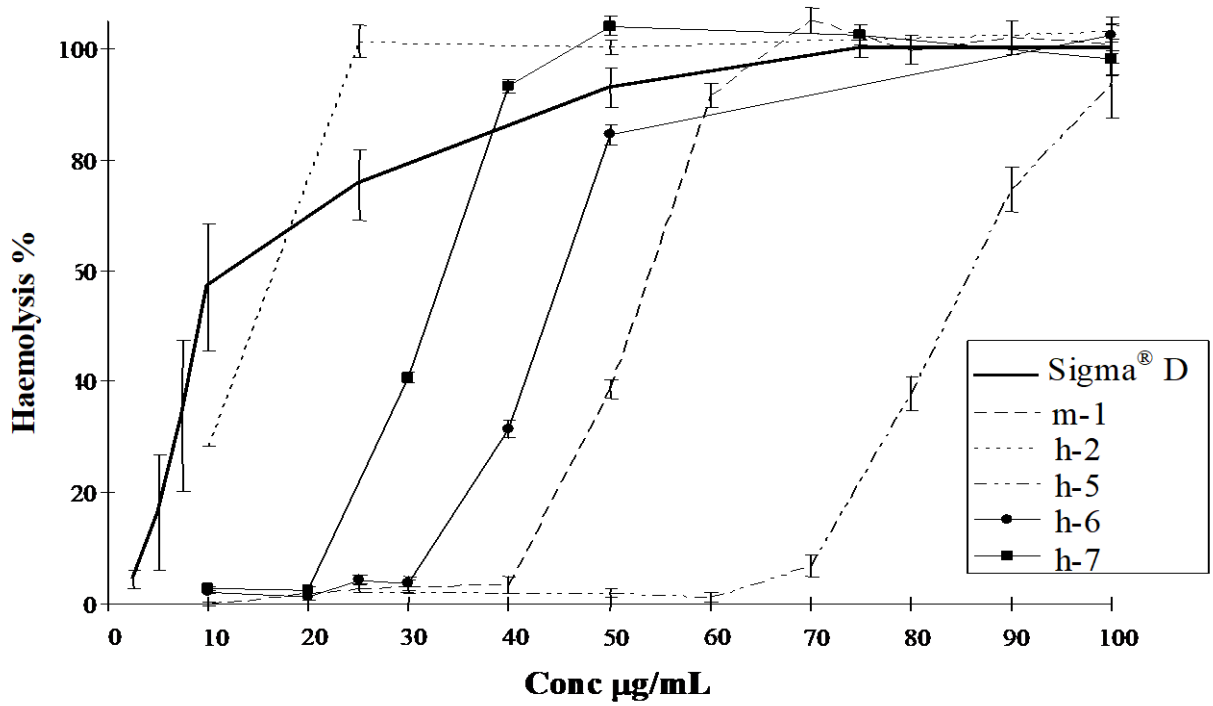


Fig. 5. Haemolytic activity of bidesmosidic saponins compared with corresponding monodesmosidic saponins.