Pergamon 0031-9422(95)00552-8 Phytochemistry, Vol. 41, No. 1, pp. 269–277, 1996

0031-9422/96 $15.00 + 0.00

TRITERPENOID SAPONINS FROM *MAESA LANCEOLATA**

JEAN B. SINDAMBIWE, ALIOU M. BALDÉ, TESS DE BRUYNE, LUC PIETERS,† HILDE VAN DEN HEUVEL, MAGDA CLAEYS, DIRK A. VAN DEN BERGHE and ARNOLD J. VLIETINCK

Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium

(*Received in revised form* 11 *April* 1995)

Key Word Index—*Maesa lanceolata*; Myrsinaceae; triterpenoid saponins; virucidal activity.

Abstract—Six new homologous triterpenoid saponins were isolated from the methanol extract of the leaves of *Maesa lanceolata* and characterized as 3β-O-[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)]-[β-D-galactopyranosyl(1 → 2)]-β-D-glucuronopyranosides of 22α-angeloyloxy-13β,28-oxydo-16α-propanoyloxyolean-21β,28α-diol, 22α-angeloyloxy-16α-butanoyloxy-13β,28-oxydoolean-21β,28α-diol, 16α,22α-diangeloyloxy-13β,28-oxydoolean-21β,-28α-diol, 22α-angeloyloxy-13β,28-oxydo-16α-(2-methyl-butanoyloxy)-olean-21β,28α-diol, 21β-acetoxy-22α-angeloyloxy-13β,28-oxydo-16α-propanoyloxyolean-28α-ol, 21β-acetoxy-22α-angeloyloxy-16α-butanoyloxy-13β,28-oxydoolean-28α-ol. The structures were established on the basis of chemical and spectral evidence.

INTRODUCTION

Within the framework of our research on natural anti-infectious agents, investigations were conducted on *Maesa lanceolata* Forsskal var. *Golungensis* (Myrsinaceae), a shrub or small tree growing in many African countries [2, 3]. The plant is well known in Rwandan traditional medicine and aqueous extracts of its leaves or fruit are parts of preparations used against various diseases including infectious hepatitis, bacillary dysentery, impetigo, ozena, some types of dermatoses and neuropathies [4]. *Maesa lanceolata* is also used to prevent cholera in East African folk medicine [5]. Two other *Maesa* species, *M. chisia* and *M. indica*, have been reported to exert antiviral activity against Rhaniket disease and vaccinia viruses [6].

The preliminary antibacterial and antiviral screening of the methanol extract from *M. lanceolata*, performed according to methods described previously [7], showed a virucidal effect on herpes simplex type 1, vesicular stomatis T2, semliki forest A7, and measles Edmonston A viruses, at 500 $\mu g\,ml^{-1}$.

Using a bioassay-guided fractionation, a saponin mixture exhibiting a moderate virucidal activity was isolated. The saponin mixture consisted of six homologous triterpenoid saponins, identified by ^1H-, ^{13}C-, and two-dimensional NMR spectroscopy and mass spectrometry. The present paper describes the isolation and the structure elucidation of the saponins.

*Part 10 in the series Plant Antiviral Agents. For part 9 see ref. [1].

†Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

The methanol extract of dried leaves of *M. lanceolata* was defatted with *n*-hexane and then partitioned between *n*-butanol and water. The *n*-butanol-soluble fraction was repeatedly subjected to silica gel and sephadex column chromatography to produce a saponin mixture, showing a reddish-purple reaction with Liebermann–Burchard reagent and forming a stable foam when shaken with water.

The aglycone mixture obtained upon acidic hydrolysis of the saponin mixture was analysed in the positive-ion FABMS mode using *m*-nitrobenzyl alcohol (*m*-NBA) saturated with lithium iodide as liquid matrix. The positive ion FAB-mass spectrum clearly revealed a mixture of aglycones showing $[M + Li]^+$ ions at *m/z* 633, 647, 659, 661, 675, and 689, corresponding to M_r values of 626, 640, 652, 654, 668, and 682, respectively. The high-mass region also contained ions at *m/z* 541, 559, 615, and 629, of which the ions at *m/z* 541 and 559 could be identified as common fragment ions of the $[M + Li]^+$ ions at *m/z* 633, 647, 659, and 661. The ions at *m/z* 615 and 629 could be interpreted as fragment ions formed by loss of CH_3COOH from the $[M + Li]^+$ ions at *m/z* 675 and 689, respectively (*vide infra*).

In order to obtain structural information on the various $[M + Li]^+$ ions, tandem mass spectrometric experiments were performed, more specifically, product ion spectra were obtained without applying collisional activation and using B/E-linked scanning. A summary of structurally useful mass spectral data is given in Table 1.

Major product ions correspond to the spontaneous loss of R_1COOH, H_2O, angelic acid, acetic acid and combinations thereof. The product ion spectra obtained for the $[M + Li]^+$ ions at *m/z* 675 and 689 clearly

Table 1. Structurally informative product ions detected in the product spectra of $[M + Li]^+$ ions of the aglycone mixture

Aglycone	M_r	$[M + Li]^+$ (*m/z*)	Product ions (*m/z*)	Rationalization (loss of)
1a	626	633	559	74 (C_2H_5COOH)
			541	74 + 18 (H_2O)
			459	74 + 100 (C_4H_7COOH)
2a	640	647	559	88 (C_3H_7COOH)
			541	88 + 18 (H_2O)
			459	88 + 100 (C_4H_7COOH)
3a	652	659	559	100 (C_4H_7COOH)
			459	100 + 100 (C_4H_7COOH)
4a	654	661	559	102 (C_4H_9COOH)
			459	102 + 100 (C_4H_7COOH)
5a	668	675	615	60 (CH_3COOH)
			515	60 + 100 (C_4H_7COOH)
6a	682	689	629	60 (CH_3COOH)
			529	60 + 100 (C_4H_7COOH)

revealed the loss of CH_3COOH, which is consistent with the presence of an acetate group.

In additional experiments, high-resolution measurements were performed on the $[M + Li]^+$ ions at *m/z* 633 using polyethylene-600 as reference and liquid matrix. The accurate mass data were consistent with an elemental composition of $C_{38}H_{58}O_7Li$ of the homologue containing a C_2H_5COO- group.

The aglycone mixture was chromatographed on silica gel and yielded a series of six compounds (**1a–6a**). ^1H NMR (at 200 and 400 MHz), ^{13}C-[Attached Proton Test(APT) mode] NMR (50 MHz) and ^1H–^1H homonuclear correlated (COSY) (400 MHz) spectra were recorded in $CDCl_3$. ^1H NMR spectral data and characteristic ^1H–^1H homonuclear correlations are given in Table 2, and ^{13}C NMR spectral data are summarized in Table 3.

The ^1H NMR spectrum of **1a** showed seven tertiary methyl protons at δ0.71, 0.78, 0.91, 0.95, 0.98, 1.08, 1.30 (3H each, *s*). Two additional signals observed at δ1.85 (3H, *s*, α-methyl) and 1.97 (3H, *d*, $J = 6.6$ Hz, β-methyl), and one typical quartet at δ6.08 (1H, *q*, $J = 6.6$ Hz) revealed the presence of an angeloyl function. This was supported by ^{13}C NMR peaks at δ127.6 (quaternary C), 138.5 (methine), 168.5 (ester carbonyl group), 20.6 and 15.5 (methyl resonances) [8–11]. ^{13}C NMR signals at δ173.5 (ester carbonyl), 28.5 (methylene) and 8.9 (methyl) indicated a propanoyloxy substituent. The ^1H NMR signals due to the propanoyloxy group overlapped those of the triterpenyl moiety. One aldehyde singlet was found at δ9.41 (1H, *s*, H-28) in the ^1H NMR spectrum and at δ201.8 in the ^{13}C NMR spectrum. This is consistent with the data reported on the aldehyde group in primulanin, which was isolated from *Primula denticulata* [12]. The ^{13}C NMR signals at δ125.4 (methine) and at 139.3 (quaternary C), together with a broad singlet at δ5.91 in the ^1H NMR spectrum, which integrates for 1H, are in agreement with a Δ^{12}-double bond and can readily be assigned to C-12, C-13, and H-12, respectively. The ^{13}C NMR signals at δ35.4, 36.9, 38.7, 39.7, 41.2, and 57.3 suggested the presence of six sp^3 quaternary carbons, as shown for mimonoside C [13]. The ^1H NMR spectrum showed a pair of doublets (AB system) at δ5.21 and 3.82 (1H, each, $J = 9.9$ Hz) that could be attributed to two vicinal methine carbons, bearing an acyloxy and a hydroxyl substituent, respectively, and having a *trans* stereochemistry. These signals are strongly correlated in the ^1H–^1H COSY spectrum. This suggested a 21-hydroxy-22-angeloyloxy substitution, as found in the pentacyclic triterpenyl angelate isolated from *Loeselia mexicana* by Jiménez *et al.* [14], which shows similar chemical shift values, and was confirmed by considerations (*vide infra*). This substitution pattern is confirmed by homonuclear ^1H–^1H correlations between H-21 and one of H-19 at δ2.41, which in turn is correlated with signals at δ2.82 (assignable to the other H-19) and δ1.37 (due to H-18, as reported in [14]). The H-22 signal at δ5.21 is not only correlated with H-21 at δ3.82, but also a multiplet at δ5.49. Therefore, the latter signal is assigned to H-16, and a second acyloxy substituent is positioned at C-16. The multiplet at δ5.49 is in turn correlated with one of H-15 at δ1.87. The multiplet at δ3.21 is assigned to H-3, which is correlated with H-2 at δ1.60. H-12 is correlated with H-11 (δ1.91). Other ^{13}C NMR assignments for **1a** listed in Table 3, were made by comparison with known spectral data for structurally related compounds, such as aesculuside A and its acetates [10], maniladiol [15], olean-12-enes and urs-12-enes described by Doddrell *et al.* [16], protoaescigenin [17] and saponins isolated from *Anagalis arvensis* [18].

The absolute stereochemistry of the triterpenyl angelate from *L. mexicana* was determined by X-ray diffraction; because of the general similarity of the ^1H NMR data for the oxygen-bearing centres, the same configuration (3β, 16α, 21β, 22α), including the 21,22-*trans* stereochemistry, was adopted for **1a**. Hence,

Table 2. Characteristic ^{1}H NMR assignments and ^{1}H–^{1}H homonuclear correlations for **1a** ($CDCl_3$, 400 MHz).

δ [ppm]	^{1}H NMR	Correlations (^{1}H–^{1}H COSY, ppm)
9.41	1H, *s*, H-28	–
6.08	1H, *q*, J = 6.6 Hz, H-3 ang.*	H-4 ang. (1.97)
5.91	1H, *br s*, H-12	H-11 (1.91)
5.49	1H, *br s*, H-16	H-22 (5.21), H-15 (1.87)
5.21	1H, *d*, J = 9.9 Hz, H-22	H-16 (5.49), H-21 (3.82)
3.82	1H, *d*, J = 9.9 Hz, H-21	H-22 (5.21), H-19a (2.41)
3.21	1H, *m*, H-3	H-2 (1.60)
2.82	1H, *m*, H-19b	H-19a (2.41), H-18 (1.37)
2.41	1H, *m*, H-19a	H-21 (3.82), H-19b (2.82) H-18 (1.37)
1.97	3H, *d*, J = 6.6 Hz, H-4 ang.	H-3 ang. (6.08)
1.91	2H, *m*, H-11	H-12 (5.91)
1.87	1H, *m*, H-15	H-16 (5.49)
1.85	3H, *br, s*, H-5 ang.	–
1.60	2H, *m*, H-2	H-3 (3.21)
1.37	1H, *m*, H-18	H-19b (2.85), H-19a (2.41)
1.30, 1.08, 0.98, 0.95, 0.91, 0.78, 0.71; 3H each, H-30, H-29, H-27, H-26, H-25, H-24, H-23.		

*ang.: angeloyl

Table 3. ^{13}C NMR assignments for **1a** ($CDCl_3$, 50 MHz)

Carbon	δ [ppm]	Carbon	δ [ppm]	Carbon	δ [ppm]
1	38.5	15	32.9	29	29.4
2	27.1	16	70.8	30	19.4
3	78.4*	17	57.3	Angeloyl	
4	38.7	18	39.0	1	168.5
5	55.2	19	46.4	2	127.6
6	18.2	20	35.4	3	138.5
7	31.3	21	69.2	4	20.6
8	39.7	22	78.8*	5	15.5
9	46.5	23	28.0	Propanoyl	
10	36.9	24	15.5	1	173.5
11	23.4	25	15.8	2	28.5
12	125.4	26	17.0	3	8.9
13	139.3	27	26.5		
14	41.2	28	201.8		

*Assignments may be reversed.

compound **1a** is 22α-angeloyloxy-3β,21β-dihydroxy-16α-propanoyloxyolean-12-en-28-al.

The ^{1}H and ^{13}C NMR spectral data obtained for compounds **2a**–**4a** are very similar to those of **1a**, the only difference lying in the 16-hydroxyl acyl substituent, as indicated by mass spectrometry of the aglycone mixture (*vide infra*). Therefore, **2a** is 22α-angeloyloxy-16α-butanoyloxy-3β,21β-dihydroxyolean-12-en-28-al. ^{1}H and ^{13}C NMR spectroscopy suggests that the unsaturated C_4H_7CO- substituent in position C-16 is also an angeloyl residue; consequently, **3a** is 16α,22α-diangeloyloxy-3β, 21β-dihydroxyolean-12-en-28-al. Compound **4a** is the saturated analogue of **3a** or 22α-angeloyloxy 3β-21β-dihydroxy-16α-(2-methylbutanoyloxy)-olean-12-en-28-al.

The ^{1}H NMR spectra of **5a** and **6a** are different in only one important aspect, i.e. the H-21/H-22 AB system consists of doublets (J = 10.2 Hz) at δ5.80 (H-21) and 5.23 (H-22). An additional methyl resonance, caused by the acetyl group, is present at δ2.31. The appearance of the H-21 and H-22 NMR signals in related pentacyclic triterpenes bearing acyloxy and hydroxyl substituents in position C-21 and C-22 has been studied in detail by Kubelka and coworkers [19, 20].

Taking into consideration the mass spectral results, **5a** and **6a** are designated as 21β-acetoxy-22α-angeloyloxy-3β-hydroxy-16α-propanoyloxyolean-12-en-28-al and 21β-acetoxy-22α-angeloyloxy-16α-butanoyloxy-3β-hydroxyolean-12-en-28-al, respectively.

The position of the acyl substituents in **1a**–**6a** and of the aldehyde function at C-28 are supported by the EI-mass spectra. The EI spectrum obtained for the aglycone mixture was interpreted with the aim of supporting partial structures, including two sets of characteristic fragment ions derived from a retro-Diels–Alder (RDA) fragmentation in the C ring of an olean-12-ene skeleton [21]. The high-mass region showed fragment peaks (relative abundance > 5%) at m/z 466, 452, 434, 424, 406, and 344. The low-mass region showed abundant characteristic fragment ions at m/z 83, 190, 198, and 207. The rationalization of structurally informative EI-mass spectral peaks is approached in Fig. 1. The ions at m/z 190 can be explained following ionization at the 3-hydroxy group, elimination of water and RDA fragmentation in the C ring. These ions could be considered as characteristic of a C-12 unsaturation in the C ring, as well as of the part of the molecule containing rings A and B. The ions at m/z 207 can be rationalized following ionization of the C-12 double bond, a series of homolytic cleavages induced by the radical centre at

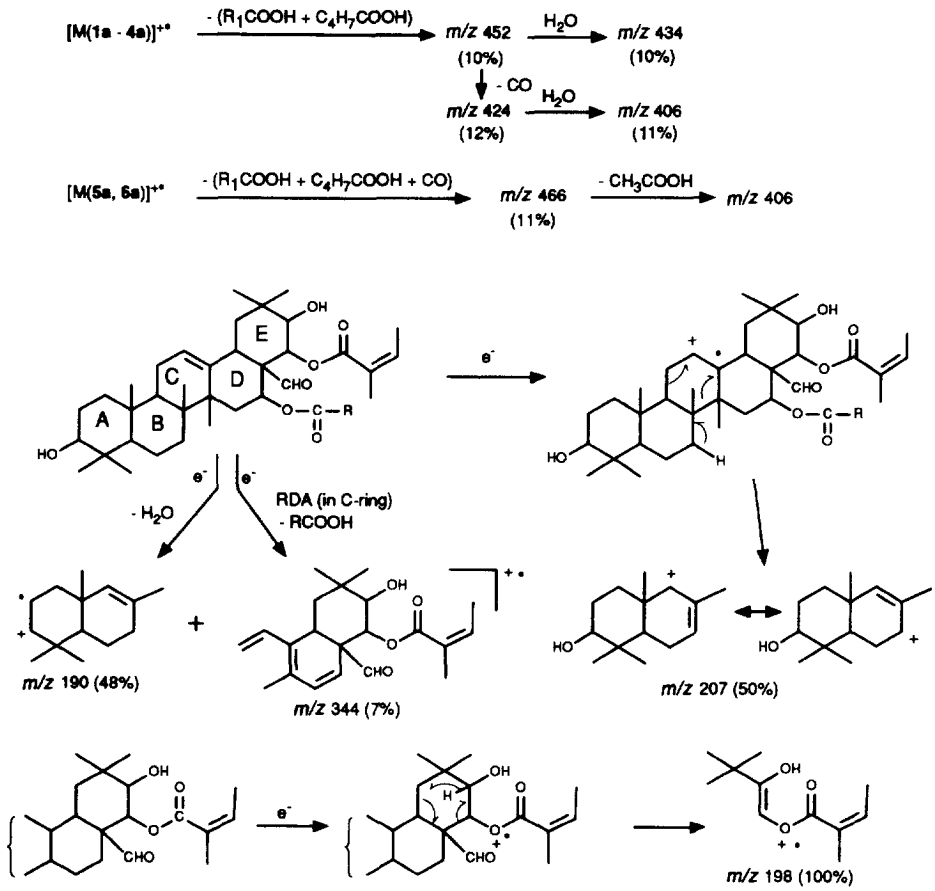


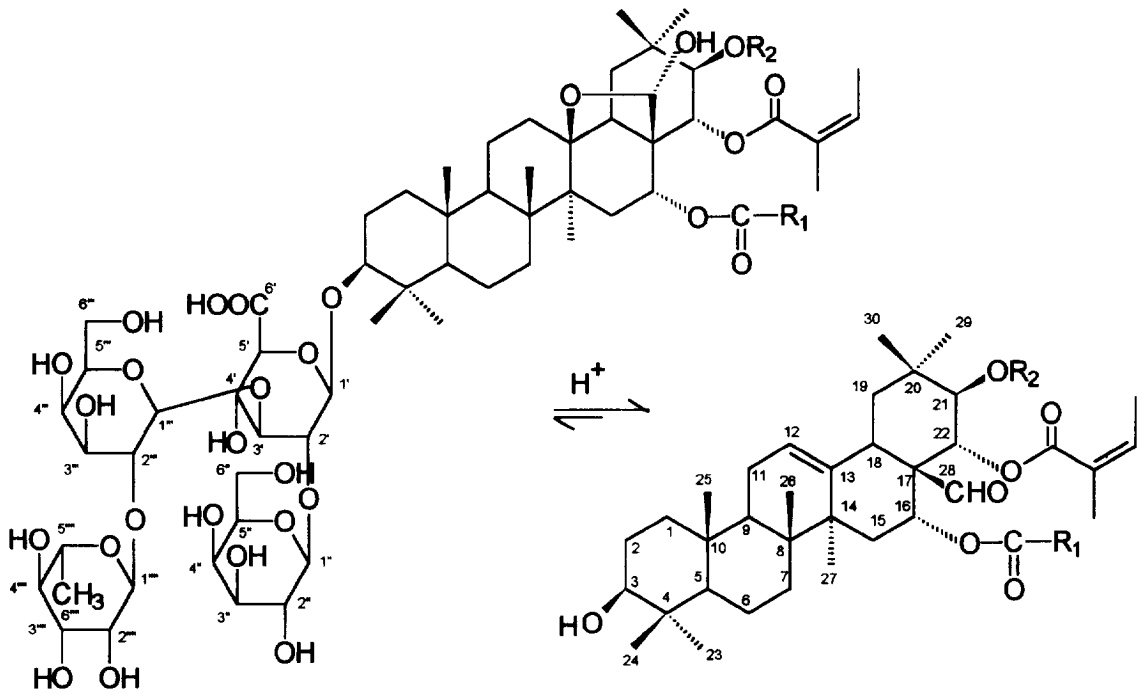
Fig. 1. Fragment ions observed in the EI-mass spectrum of **1a**–**6a**.

C-13, i.e. cleavage of the 8, 14-bond and the [C-7] H-bond, and heterolytic cleavage of the 9, 11-bond, resulting in resonance-stabilized allylic ions. As holds for the ions at *m/z* 190, the ions at *m/z* 207 could be regarded as structurally informative, since they are consistent with a C-12 unsaturation and contain the AB-ring part of the molecule. All the other fragment ions are in agreement with the proposed structures. The ion at *m/z* 83 $[C_4H_7CO]^+$ in particular corroborates the presence of an angeloyl group. The ion at *m/z* 198 can be interpreted as a H rearrangement in the E ring as proposed in Fig. 1 and provides supporting evidence for the occurrence of an angeloyl substituent in the E ring. The ion at *m/z* 344 can be explained by a retro-Diels–Alder fragmentation in the C ring and loss of RCOOH, and are consistent with the presence of an RCOO- substituent in the D ring.

The ^1H and ^{13}C NMR spectra of the saponin mixture (**1**–**6**) indicated that all aglycones were substituted at position C-3 by the same tetrasaccharidic moiety, consisting of D-glucuronic acid, L-rhamnose, and two units of D-galactose. Four anomeric carbon signals were indeed observed at δ105.61, 102.25, 103.22, and 100.95.

The same sugars were identified in the aqueous fraction obtained after acidic hydrolysis, by comparison with authentic sugar reference samples on TLC. In the ^{13}C NMR spectrum, the C-3 signal was shifted downfield by almost 14 ppm to δ92.04, characterizing the glycosylation site. New signals were noticed in the ^{13}C NMR spectrum at δ97.41 (methine, C-28), and 88.81 (quaternary C,C-13). ^1H and ^{13}C NMR signals typical of the Δ^{12}-double bond and of the aldehyde group in the aglycones, were not present at all. The ^1H NMR spectrum showed a peak at δ4.69 (1H, *s*, H-28). This is in agreement with the presence of a 13,28-oxido moiety containing a hemiacetal function at C-28, as described for an oleane triterpene from *Anagalis arvensis* [22, 23], and triterpene saponins from *P. veris* [24]. Upon acidic hydrolysis, the latter compounds form a Δ^{12}-double bond and a C-28 aldehyde [25]. These results imply that the aglycones obtained are not the genuine ones from the original saponins and correspond to stable derivatives.

Upon FABMS using *m*-NBA as liquid matrix, a negative-ion fast atom bombardment (FAB) spectrum was obtained, showing a series of $[M-H]^-$ ions at *m/z* 1289, 1303, 1315, 1317, 1331, and 1345, indicating M_r values of 1290, 1304, 1316, 1318, 1332, and 1346, respectively. Complementary M_r information was derived from the positive ion FAB mass spectrum obtained using *m*-NBA saturated with lithium iodide as liquid matrix, which showed $[M - H + 2Li]^+$ ions at *m/z* 1303, 1317, 1329,



Saponin	R_1	R_2	Amount (%)	Aglycone
1	C_2H_5	H	15	**1a**
2	C_3H_7	H	15	**2a**
3	C_4H_7	H	8	**3a**
4	C_4H_9	H	12	**4a**
5	C_2H_5	CH_3CO	31	**5a**
6	C_3H_7	CH_2CO	19	**6a**

Fig. 2. Chemical structure of **1–6** and **1a–6a**, and relative composition of **1–6**.

1331, 1345 and 1359. The formation of $[M - H + 2Li]^+$ ions is consistent with the occurrence of an acidic group in the carbohydrate moiety. The relative composition of the saponin mixture given in Fig. 2 was estimated based on the relative abundance of $[M - H]^-$ ions. In addition to $[M - H]^-$ ions, the negative-ion FAB mass spectrum also revealed fragment ions indicative of terminal hexose (162 units loss) and hexose-deoxyhexose (308 units loss) residues.

In an effort to obtain more specific sequence information on the carbohydrate moiety, the acylated saponin mixture was examined by positive ion FAB mass spectroscopy using *m*-NBA as liquid matrix. Fragmentions were observed at *m/z* 273, 331, 561 and 1131, consistent with a terminal rhamnopyranosyltriacetate and galactopyranosyltetraacetate, a rhamnonopyranosyltriacetate-galactopyranosyltriacetate, and a glucuronopyranosyl-monoacetate-containing tetrasaccharide moiety, respectively. The formation of these fragment ions is outlined in Fig. 3 and can be explained in a straightforward manner following protonation of the interglycosidic bonds. The same reaction as outlined for the formation of the *m/z* 273 ions occurs at the other interglycosidic linkages. The glucuronopyranosyl moiety was found to correspond to the innermost sugar unit. From these mass spectral data, the sequence [(rhamnopyranosyl-galactopyranosyl)-(galactopyranosyl)-glucuronopyranosyl]-aglycone could be established unambiguously.

In order to complete the structure characterization of the glycosidic part of the saponins, ^1H and ^{13}C NMR, DEPT, ^1H–^1H COSY, and ^1H–^{13}C heteronuclear HMQC and HMBC were recorded in CD_3OD on a 600 MHz instrument. Long-range ^1H–^{13}C correlations between each anomeric proton across the glycosidic bond and a carbon atom of another substituted sugar unit or the aglycone were used to determine the sugar sequence. Characteristic ^1H NMR assignments, homonuclear ^1H–^1H and heteronuclear ^1H–^{13}C long-range correlations are listed in Table 4, ^{13}C NMR assignments of the glycosidic moiety are given in Table 5. The C-3 signal at δ92.08 is readily identified as outlined above. This signal is directly correlated with a multiplet at δ3.20 in the ^1H NMR spectrum (1H, *m*, H-3), and shows a long-range correlation with the signal at δ4.53 (1H, *d*, J = 7.4 Hz), which in turn is directly correlated with the anomeric ^{13}C NMR peak at δ105.61. Hence, the latter signals are attributed to H-1 and C-1 of the glucuronic acid moiety, respectively, which was shown by mass

spectral considerations to be directly connected to the aglycone. The ^{13}C NMR signal δ105.61 exhibits a long-range correlation with the ^{1}H NMR peak at δ3.93, which is assigned to H-2′. This is confirmed by a ^{1}H–^{1}H correlation between H-1′ and H-2′. The multiplet at δ3.93 (H-2′) is directly correlated with the ^{13}C NMR signal at δ79.44 (C-2′), and shows a long-range correlation with the anomeric ^{13}C NMR peak at δ103.22. As the latter signal is directly correlated with a ^{1}H NMR doublet at δ4.83 (J = 7.4 Hz), these signals are attributed to C-1″ and H-1″ of one of the galactose moieties (gal I), respectively. The C-2′ signal at δ79.44 also shows a long-range correlation with the ^{1}H NMR signal at δ4.14 (H-3′). In addition, a ^{1}H–^{1}H correlation between H-2′ and H-3′ is observed. The ^{1}H NMR multiplet at δ4.14 is directly correlated with the ^{13}C NMR peak at δ81.58, which suggests a glycosidic substitution at this position. This is confirmed by a long-range correlation between the ^{1}H NMR multiplet at δ4.14 (H-3′) and the anomeric ^{13}C NMR signal at δ100.95, directly correlated with a doublet in the ^{1}H NMR spectrum at δ5.24 (J = 7.4 Hz); the latter signals are assigned to C-1‴ and H-1‴ of the second galactose moiety (gal II), respectively. Another long-range correlation of the multiplet at δ4.14 with the ^{13}C NMR peak at δ72.44 (C-4′), directly correlated with a ^{1}H NMR signal at δ3.65 (H-4′), lead to the assignment of C-4′ and H-4′, which is confirmed by a ^{1}H–^{1}H correlation between H-3′(δ4.14) and H-4′ (δ3.65). The ^{1}H NMR signal at δ3.74 shows long-range correlations with the carbon atoms resonating at δ72.44 (C-4′) and δ176.74 (C-6′), and is attributed to H-5′; it is directly correlated with a ^{13}C NMR signal at δ77.11 (C-5′). The results indicate that all the ^{1}H and ^{13}C NMR signals of the glucuronic acid moiety could be assigned. The anomeric ^{13}C NMR peak of gal II at δ100.95 (C-1‴) shows a long-range correlation with a ^{1}H NMR multiplet at δ3.81, assigned to H-2‴, which is directly correlated to a ^{13}C NMR signal at δ76.84, assigned to C-2‴ (coinciding with C-5′). The position of the rhamnose unit at C-2‴ of gal II is indicated by a long-range correlation of its anomeric ^{13}C NMR signal at δ102.25 (C-1⁗) directly correlated with a broad singlet in the ^{1}H NMR spectrum at δ5.29 (H-1⁗), with H-2‴ (δ3.81). This is supported by a long-range correlation of H-1⁗ with C-2‴ (δ76.84). Therefore, the glycosidic linkage sites, which are in agreement with the mass spectral results, are determined unambiguously. Hence, the structure of the saccharide moiety is [α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)]-[β-D-galactopyranosyl(1 → 2)]-β-D-glucuronopyranoside. The other assignments in Tables 4 and 5 are based upon correlations observed in the

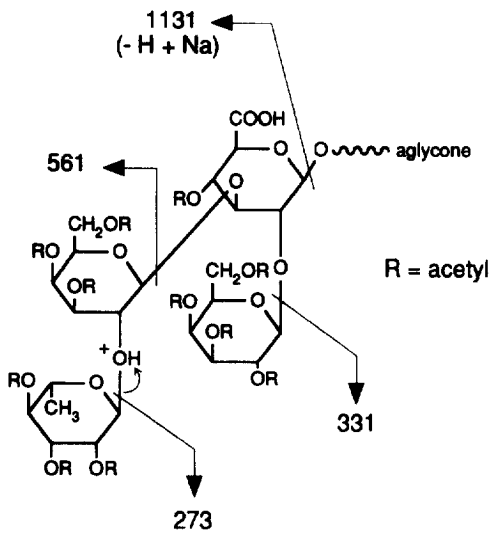


Fig. 3. Fragment ions obtained for the acetylated saponin mixture by positive ion FAB-mass spectrometry.

Table 4. Characteristic ^{1}H NMR assignments (J in Hz), homonuclear ^{1}H–^{1}H and heteronuclear ^{1}H–^{13}C long-range correlations for the glycosidic part of **1**–**6** (CD_3OD, 600 MHz)

	H	^{1}H NMR	^{1}H–^{1}H (COSY)	^{1}H–^{13}C (long-range)
Aglycon.	3	3.20, *m*	–	–
GlcA	1′	4.53, *d*, J = 7.4	H-2′ (δ3.93)	C-3 (δ92.08)
	2′	3.93, *m*	H-1′ (δ4.53), H-3′ (δ4.14)	C-1′ (δ105.61), C-3′ (δ81.58), C-1″ (δ103.22)
	3′	4.14, *m*	H-2′ (δ3.93), H-4′ (δ3.65)	C-2′ (δ79.44), C-4′ (δ72.44), C-1‴ (δ100.95)
	4′	3.65, *m*	H-3′ (δ4.14), H-5′ (δ3.74)	C-3′ (δ81.58), C-5′ (δ77.11)
	5′	3.74, *m*	H-4′ (δ3.65)	C-4′ (δ72.44), C-6′ (δ176.74)
	6′	–	–	–
Gal I	1″	4.83, *d*, J = 7.4	H-2″ (δ3.62)	–
	2″	3.62, *m*	H-1″ (δ4.83)	C-1″ (δ103.22), C-3″ (δ74.91)
Gal II	1‴	5.24, *d*, J = 7.4	H-2‴ (δ3.81)	–
	2‴	3.81, *m*	H-1‴ (δ5.24)	C-1‴ (δ100.95), C-3‴ (δ75.85), C-1⁗ (δ102.25)
Rha	1⁗	5.29, *br s*,	H-2⁗ (δ3.98)	C-2⁗ (δ72.35), C-5⁗ (δ70.13), C-2‴ (δ76.84)
	2⁗	3.98, *m*	H-1⁗ (δ5.29)	–
	5⁗	4.14, *m*	H-6⁗ (δ1.31)	–
	6⁗	1.31, *d*, J = 6.2	H-5⁗ (δ4.14)	C-5⁗ (δ70.13)

Table 5. ^{13}C NMR assignments for the glycosidic part of **1**–**6** (CD_3OD, 175 MHz)

Carbon	δ [ppm]	Carbon	δ [ppm]
Glucuronic acid		Galactose II	
1′	105.61	1‴	100.95
2′	79.44	2‴	76.84
3′	81.58	3‴	75.84
4′	72.44	4‴	71.64
5′	77.11	5‴	76.67
6′	176.74	6‴	62.72*
Galactose I		Rhamnose	
1″	103.22	1⁗	102.25
2″	73.28	2⁗	72.35
3″	74.91	3⁗	72.28
4″	70.13	4⁗	73.80
5″	76.84	5⁗	70.13
6″	62.81*	6⁗	18.03

*assignments may be reversed.

two-dimensional NMR spectra and by comparison with known saponins containing a related glycosidic moiety, such as the triterpene saponins isolated from *Calendula arvensis* [26], *C. officinalis* [27], *Eleutherococcus senticosus* [28], *Herniaria glabra* [29], *P. veris* [24], and *Rapanea melanophloeos* [30]. Analysis of the two-dimensional NMR data also enables the assignment of some characteristic ^1H and ^{13}C NMR signals of the triterpenyl part of the saponin. If an acetyl group is present at position C-21, as for **5** and **6**, which are the main compounds of the saponin mixture, H-21 is observed as a doublet at δ6.03 ($J = 9.9$ Hz). H-21 is directly correlated with the C-21 signal at δ80.15, and shows a ^1H–^1H correlation with a doublet at δ5.52 ($J = 9.9$ Hz), assigned to H-22 and directly correlated with C-22 at δ74.40. The peaks of the acetyl group appear at δ171.41 (carbonyl) and δ21.90 (methyl) in the ^{13}C NMR spectrum, and at δ2.31 (methyl) in the ^1H NMR spectrum. The ^{13}C NMR signal at δ97.41 (C-28) correlates directly with a singlet at δ4.69 (H-28) in the ^1H NMR spectrum.

The NMR and mass spectral data interpreted above, as well as the chemical evidence, allow us to identify compounds **1**–**6** in the unresolved saponin mixture (relative composition, see Fig. 2) as follows:

1: 3β-*O*-{[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)]-[β-D-galactopyranosyl(1 → 2)]-β-D-glucuronopyranosyl}-22α-angeloyloxy-13β,28-oxydo-16α-propanoyloxyolean-21β,28α-diol,

2: 3β-*O*-{[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)]-[β-D-galactopyranosyl(1 → 2)]-β-D-glucuronopyranosyl}-22α-angeloyloxy-16α-butanoyloxy-13β,28-oxydoolean-21β,28α-diol,

3: 3β-*O*-{[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)]-[β-D-galactopyranosyl(1 → 2)]-β-D-glucuronopyranosyl}-16α,22α-diangeloyloxy-13β,28-oxydoolean-21β,28α-diol,

4: 3β-*O*-{[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)]-[β-D-galactopyranosyl(1 → 2)]-β-D-glucuronopyranosyl}-22α-angeloyloxy-13β,28-oxydo-16α-(2-methylbutanoyloxy)-olean-21β,28α-diol,

5: 3β-*O*-{[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)]-[β-D-galactopyranosyl(1 → 2)]-β-D-glucuronopyranosyl}-21β-acetoxy-22α-angeloyloxy-13β,28-oxydo-16α propanoyloxyolean-28α-ol,

6: 3β-*O*-{[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)]-[β-D-galactopyranosyl(1 → 2)]-β-D-glucuronopyranosyl}-21β-acetoxy-22α-angeloyloxy-16α-butanoyloxy-13β,28-oxydoolean-28α-ol.

EXPERIMENTAL

General experimental procedures. CC performed on silica gel 60, Merck (230–400 mesh ASTM) and Sephadex LH-20, Pharmacia (5–100 μm), using as eluent systems: *n*-BuOH:HOAc:H_2O (4:1:5), upper layer (a), *n*-BuOH:EtOH:H_2O (4:1:2.2) (b), MeOH (c), and a gradient of $CHCl_3$–MeOH from $CHCl_3$ to $CHCl_3$–MeOH (1:1) (d). In order to monitor the elution, analytical TLC was carried out on precoated silica gel 60 F_{254} plates, Merck (layer thickness 0.2 mm); the plates were developed in solvent system (a) for saponins and sapogenins and sprayed with Liebermann–Burchard reagent or 1% $FeCl_3$ in 0.05 M HCl, while sugars were eluted with $CHCl_3$:MeOH:H_2O (6.4:4.0:0.8) (e) and revealed with 1-naphthol-H_2SO_4 for sugars (Merck).

Mass spectra were recorded on a Fisons V670SEQ mass spectrometer, equipped with a caesium ion. The acronym FAB (fast atom bombardment) is used in this study to refer to caesium ion bombardment. Caesium ions with an impact energy of 18 KeV (+ ion mode) or 25 keV (− ion mode) were used as the ionization beam. Accelerating voltage in the source was 8 kV. EI mass spectra were obtained using ionization with 70 eV electrons.

^1H and ^{13}C NMR spectra were recorded on Jeol FX 200 (200 MHz), Varian Unity (400 MHz), and Bruker AM 600 (600 MHz) instruments. Chemical shifts are reported in δ values downfield from internal TMS. All one-dimensional and two-dimensional-NMR spectra were recorded using standard instrumental software packages. Two-dimensional COSY and ^1H-detected one-bond (HMQC) and multiple-bond (HMBC) ^{13}C multiple-quantum coherence spectra, recorded on the 600 MHz instrument, were obtained using the conditions reported previously [31].

Plant material. Maesa lanceolata leaves were collected in Butare, Rwanda, in August 1989. The plant material was identified by Dr J. Mvukiyumwami, Botany Department, Institut de la Recherche Scientifique et Technique (IRST). A voucher specimen is preserved in the herbarium of IRST. The leaves were oven-dried at 40° and then ground.

Extraction and isolation of Maesa *saponins.* Dried and powdered leaves of *M. lanceolata* (1 kg) were exhaustively extracted with aq. MeOH (90%). The MeOH extract was evaporated *in vacuo* to provide a viscous dark-brown residue (460 g), which was suspended in water and successively extracted with *n*-hexane and *n*-BuOH. A

bioassay-guided fractionation was started in order to isolate the active compounds. The *n*-BuOH extract (20 g portions) was repeatedly subjected to column chromatography on 400 g silica gel, which was eluted with (a) at a flow rate of 10 ml hr^{-1}. Fractions (15 ml) were collected and similar ones were combined on the basis of their silica gel TLC profiles. The anti-herpes simplex type 1 virus active (virucidal) fraction, tested as described previously [7], was further chromatographed on silica gel and Sephadex columns, eluted with (b) and (c), respectively. A saponin powder (10 g), showing a reddish-purple reaction in the Liebermann–Burchard test or whitish spots with $FeCl_3$ and forming a stable foam when shaken with water, was obtained. The saponin mixture isolated showed a moderate virucidal activity against enveloped viruses.

Acidic hydrolysis. The saponin sample (200 mg) was dissolved in a minimal volume of $HCl:H_2O:EtOH$ (2:1:2) and hydrolysed by refluxing on a boiling water bath for 30 min. The hydrolysate was allowed to cool, diluted twofold with distilled H_2O and partitioned between water and EtOAc. The aq. layer was concentrated *in vacuo* and compared with standard sugars by silica gel TLC, using eluent system (e) and spraying with 1-naphthol-H_2SO_4. D-glucuronic acid, D-galactose, and L-rhamnose were identified. The carbohydrate sequence was established by MS and further structural features were inferred from ^1H and ^{13}C NMR, DEPT, ^1H–^1H COSY and ^1H–^{13}C heteronuclear HMQC and HMBC data. The EtOAc layer was evaporated *in vacuo* to yield 125 mg of sapogenin mixture (63%), which was resolved into a series of aglycones on a silicagel column, using (d) as eluent. The aglycones **1a–6a** were identified by MS, ^1H, ^{13}C and two dimensional-NMR, including ^1H–^1H chemical shift correlation spectroscopy (COSY).

1a–6a: Pos. FABMS (Table 1). **1a–4a**: EIMS *m/z* (rel. int.):452 (10) $[M - (R_1COOH + C_4H_7COOH)]^+$, 434 (10) $[452–H_2O]^+$, 424 (12) $[M - (R_1COOH + CO + C_4H_7COOH)]^+$, 406 (11) $[424 - H_2O]^+$, 344 (7) [(M – R_1COOH), RDAb]$^+$, 207 (50) [RDAa]$^+$, 198 (100) (see Fig. 4), 190 (48) [(M – H_2O), RDAa]$^+$. **5a, 6a**: EIMS *m/z* (rel. int.): 466 (11) $[M - (R_1COOH + CO + C_4H_7COOH)]^+$, 406 $[466 - CH_3COOH]^+$; ^1H NMR ($CDCl_3$, 400 MHz): δ5.80 (1H, *d*, *J* = 10.2 Hz, H-21), 5.23 (1H, *d*, *J* = 10.2 Hz, H-22), 2.31 (3H, *s*, CH_3CO). **1a**: ^1H–NMR ($CDCl_3$, 400 MHz) (Table 2); ^{13}C NMR ($CDCl_3$, 50 MHz) (Table 3).

1–6: Neg. FABMS: $[M - H]^-$ *m/z* 1289, 1303, 1315, 1317, 1331, 1345; complementary pos. FABMS: $[M - H + 2Li]^+$ *m/z* 1303, 1317, 1329, 1331, 1345, 1359; *m/z* 981 $[M - H - 308]^-$, 995 $[M - H - 308]^-$. 1007 $[M - H - 308]^-$, 1009 $[M - H - 308]^-$, 1023 $[M - H - 308]^-$, 1037 $[M - H - 308]^-$; 1129 $[M - H - 162]^-$, 1143 $[M - H - 162]^-$, 1153 $[M - H - 162]^-$, 1155 $[M - H + 2Li - 162]^+$, 1169 $[M - H + 2Li - 162]^+$, 1185 $[M - H + 2Li - 162]^+$; glycosidic part: ^1H NMR (CD_3OD, 600 MHz) (Table 4); ^{13}C NMR (CD_3OD, 175 MHz), (Table 5).

5–6: ^1H NMR (CD_3OD, 600 MHz): δ6.03 (1H, *d*, *J* = 9.9 Hz, H-21), 5.52 (1H, *d*, *J* = 9.9, H-22), 4.69 (1H, *s*, H-28), 2.31 (3H, *s*, CH_3CO); ^{13}C NMR (CD_3OD, 175 MHz): δ175.55 (COR), 171.41 ($COCH_3$), 169.19 (angeloyl C=0), 139.30 (angeloyl C-3), 129.20 (angeloyl C-2); 97.41 (C-28), 92.08 (C-3), 88.81 (C-13), 80.15 (C-21), 74.40 (C-22), 27.90 (CH_3CO).

Acknowledgements This work was supported by a grant from the Belgian General Agency for Development and Cooperation (ABOS/AGCD), grants of the National Fund for Scientific Research (NFWO, Belgium), the Flemish Government (Belgium) (grant No. 92/94-09), and the University of Antwerp (UIA) (fellowship 1994–96 for TDB). We are grateful to Dr V. Wray (GBF, Braunschweig, Germany) for recording the 600 MHz NMR spectra and to Dr J. Mvukiyumwami for the identification of the plant material.

REFERENCES

1. Corthout, J., Pieters, L., Claeys, M., Vanden Berghe, D. and Vlietinck, A. (1992) *Phytochemistry* **31**, 1979.
2. Troupin, G. (1982) *Flore des Plantes Ligneuses du Rwanda*, Vol. 21, p. 459. INRS, Butare.
3. Raynal, J., Troupin, G. et Sita, P. (1985) *Contribution aux Études Floristiques au Rwanda*, p. 147. ACCT, Paris.
4. Kayonga, A. et Habiyaremye, F. X. (1987) *Médecine Traditionnelle et Plantes Médicinales Rwandaises, Enquêtes Ethnobotaniques-Préf. de Gisenyi*, p. 118. UNR, Butare.
5. Kubo, I., Kamikawa, T. and Miura, I. (1983) *Tetrahedron Letters* **24**, 3825.
6. Che, C.-T. (1991) in *Economic and Medicinal Plant Research* (Wagner, H. and Farnsworth, N. R., eds), Vol. 5, pp. 167–251. Academic Press, London.
7. vanden Berghe, D. A. and Vlietinck, A. J. (1991) in *Methods in Plant Biochemistry*, (Dey, P. M. and Harborne, J. B., eds.), pp. 47–69. Academic Press, London.
8. Chakravarty, A. K., Das, B. and Pakrashi, S. C. (1987) *Phytochemistry* **26**, 2345.
9. Chen, Y., Takeda, T. and Ogihara, Y. (1985) *Chem. Pharm. Bull.* **33**, 1387.
10. Singh, B., Agrawal, P. K. and Thakur, R. S. (1986) *Planta Med.* **56**, 409.
11. Yosiyoka, I., Hino, K., Matsuda, A. and Kitagawa, I. (1972) *Chem. Pharm. Bull.* **20**, 1499.
12. Ahmad, V. U., Sultana, V., Arif, S. and Saqib, Q. N. (1988) *Phytochemistry* **27**, 304.
13. Jiang, Y., Haag-Berrurier, M., Anton, R., Massiot, G., Lavaud, C., Teulon J. M. and Guéchot, C. (1991) *J. Nat. Prod.* **54**, 1247.
14. Jiménez, M. E., Velasquez, K., Lira-Rocha, A., Ortega, A., Diaz, E., Aumelas, A. and Jankovski, K. (1989) *Can. J. Chem.* **67**, 2071.
15. Kouam, J., Nkengfack, E. A., Fomum, Z. T., Ubillas, R., Tempesta, M. S. and Meyer, M. (1991) *J. Nat. Prod.* **54**, 1288.
16. Doddrell, D. M., Khong, P. and Lewis, K. G. (1974) *Tetrahedron Letters* **27**, 2381.

17. Agrawal, P. K., Thakur, R. S. and Shoolery, J. N. (1991) *J. Nat. Prod.* **54**, 1394.
18. Amoros, M. and Girré, R. L. (1987) *Phytochemistry* **26**, 787.
19. Aurada, E., Jurenitsch, J. und Kubelka, W. (1984) *Planta Med.* **50**, 391.
20. Jurenitsch, J., Aurada, E., Robien, W. und Kubelka, W. (1984) *Sci. Pharm.* **52**, 141.
21. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Pharm. Soc.* **85**, 3688.
22. Aliotta, G., De Napoli, L., Giordano, F., Picciali, G. and Santacroce, C. (1992) *Phytochemistry* **31**, 929.
23. Glombitza, K. W. und Kurth, H. (1987) *Planta Med.* **53**, 548.
24. Çaliş, I., Yürüker, A. Ruëgger, H., Wright, A. D. and Sticher, O. (1992) *J. Nat. Prod.* **55**, 1299.
25. Kitagawa, I., Matsuda, A. and Yosiyoka, I. (1972) *Chem. Pharm. Bull.* **20**, 2226.
26. Pizza, C., Zhong-Liang, Z. and de Tommasi, N. (1987) *J. Nat. Prod.* **50**, 927.
27. Vidal-Ollivier, E., Balansard, G., Faure, R. and Badadjamian, A. (1989) *J. Nat. Prod.* **52**, 1156.
28. Segiet-Kujawa, E. and Kaloga, M. (1991) *J. Nat. Prod.* **54**, 1044.
29. Schröder, H., Schubert-Zsilavecz, M., Reznicek, G., Cart, J., Jurenitsch, J. and Haslinger, E. (1993) *Phytochemistry* **34**, 1609.
30. Ohtani, K., Mavi, S. and Hostettmann, K. (1993) *Phytochemistry* **33**, 83.
31. Schwind, P., Wray, V. and Nahrstedt, A. (1990) *Phytochemistry* **29**, 1903.