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TRITERPENOID SAPONINS FROM FRUIT OF *LAGENARIA BREVIFLORA*

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Key Word Index—*Lagenaria breviflora*; Cucurbitaceae, fruit, triterpenoid saponins

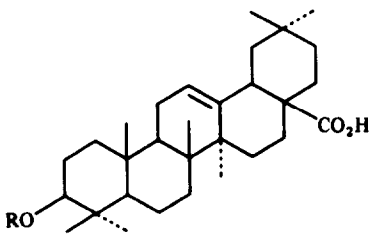
Abstract—From the methanol extract of the fruit pulp of *Lagenaria breviflora*, three new saponins were characterized as 3-*O*-β-galactopyranosyl 28-*O*-β-xylopyranosyl(1-4)-α-rhamnopyranosyl(1-3)-β-xylopyranosyl(1-3)-α-arabinopyranosylolean-12-en-28-oic acid ester, 3-*O*-β-galactopyranosyl 28-*O*-β-galactopyranosyl(1-4)-α-rhamnopyranosyl(1-3)-β-xylopyranosyl(1-3)-α-arabinopyranosylolean-12-en-28-oic acid ester and 3-*O*-β-galactopyranosyl 28-*O*-α-arabinopyranosyl(1-6)-β-galactopyranosyl(1-4)-α-rhamnopyranosyl(1-3)-β-xylopyranosyl-(1-3)-α-arabinopyranosylolean-12-en-28-oic acid ester. Oleanolic acid and 3-*O*-acetyloleanolic acid were identified from the hydrolytic products of the pulp.

INTRODUCTION

Lagenaria breviflora Robert (*Adenopus breviflorus* Benth) Cucurbitaceae, is an annual climbing plant used in Nigerian traditional medicine and whose antifertility [1, 2] and anticonvulsant [3] activities have been reported There have been no detailed phytochemical reports on the species. A phytochemical study has been undertaken and this paper describes the isolation and structural elucidation of three new triterpenoid saponins, a class of natural products to which a wide range of biological activities has been attributed [4].

RESULTS AND DISCUSSION

Direct acid hydrolysis of the powdered pulp of *L. breviflora* fruit yielded two major aglycones **1** and **2**, identified as oleanolic acid and 3-*O*-acetyloleanolic acid, respectively, by comparison with literature data [5, 6] and authentic samples (co-TLC, co-IR, EIMS, ^{1}H and ^{13}C NMR). The presence of an acetyl group in compound



	R
1	H
2	Ac
7	Gal

2 was deduced from the EI mass spectrum which indicated a molecular weight of 498 and showed a fragment at *m/z* 455 $[M-Ac]^+$. The ^{13}C NMR spectrum confirmed the presence of an acetyl group with signals at δ170.63 (MeCO) and 21.15 (MeCO) ppm. In addition, the C-3 signal in compound **2** was shifted (Table 1) to δ80.73, as opposed to 78.09 for compound **1** Deacetylation of compound **2** by mild alkaline hydrolysis gave **1** confirming its structural relationship to oleanolic acid.

The methanol extract of the fruit pulp showed seven zones on TLC, which on repeated chromatography (prep. TLC) and purification, afforded three compounds **4–6**, which foamed vigorously when shaken with distilled water [4]. On acid hydrolysis, compounds **4–6** yielded the same aglycone identified as oleanolic acid and also similar monosaccharides identified as galactose, arabinose, xylose and rhamnose by PC, GC and ^{13}C NMR (Table 2) by comparison with the literature data [7, 8]. However, the number of monosaccharides differed in each of the saponins. The ^{13}C NMR spectra of compounds **4–6** when compared to that of compound **1** (Table 1) indicated glycosidation shifts [9] at C-2, C-3, C-17 and C-28, suggesting that **4–6** are bisdesmosides of oleanolic acid having sugar linkages at both the C-3 hydroxyl and C-28 carboxyl groups. Signals at δ88.80 and 171.31 were strongly suggestive of glycosidation at the latter two positions, respectively [8] Alkaline hydrolysis of **4–6** yielded the same monodesmosides (prosapogenin) **7** (co-TLC, co-IR and FABMS). The presence of a carboxylic acid group was confirmed by a ^{13}C NMR signal at δ180.18 (Table 1) The FAB mass spectra of compound **7** showed fragment ions at 641 $[M+Na]^+$ and 477 $[M+Na-Gal]^+$ confirming that only one hexose (identified as galactose by PC of the acid hydrolysate and ^{13}C NMR of **7**) was attached to the C-3 of oleanolic acid.

Compounds **4** and **5** (pentaglycosides) showed five anomeric signals in their ^{13}C NMR spectra whilst compound **6** (hexaglycoside) showed six. The anomeric signal *ca* δ93.4 showed that the arabinosyl residue was attached directly to C-28 [10] in all these three saponins. The C-28 terminal xylose in **4** and terminal arabinose in **6** gave

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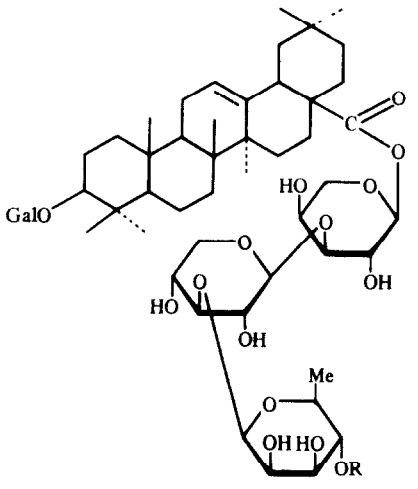
Table 1 ^{13}C NMR chemical shifts of aglycone moieties of compounds **1**, **2**, **4**, **6**–**10** (in pyridine-d_5)

C	1	2	4	6	7	8	9	10
1	38 96	38 15	38 74	38 75	38 80	38 52	38 58	37 03
2	28 14	30 02	26 59	26 61	25 26	29.75	29.63	26 04[a]
3	78 09	80 73	88 87	88 86	88 80	89 78	89.83	98 59
4	39 42	37 88	39 53	39 53	39 51	39 20	39 10	39 15
5	55 84	55 51	55 84	55 82	55 79	55 65	55 65	55 71
6	18 82	18 48	18 59	18 57	18 30	17 48	17 45	18 53
7	33 23[a]	33 20[a]	32 73[a]	32 79[a]	32 20[a]	32 40	32 41	30 87[b]
8	39 79	39 69	39 88	39 88	39 75	39.85	39 85	39 93
9	48 15	47 88	47 98	47 99	47 99	47 96	48 01	48 04
10	37 41	37 16	36 98	36 98	37 38	37 01	36 97	37 03
11	23 79[b]	23 68[b]	23 81[b]	23.22	23 77[b]	23 97[a]	23 79[a]	23 84[c]
12	122 61	122 39	122.76	122 76	122 57	122 75	122 75	122 99
13	144 86	144 86	144.21	144 21	144.84	144 83	143 97	144 95
14	42 20	42 14	42.12	42 12	42.17	42 23	42 25	42 27
15	28 35[c]	28 30[c]	29 90	28 31[b]	28.31[c]	26 29	26 27	27 97[d]
16	23 85[b]	23 89[b]	23 19	23 22	22 95	23 77[a]	23 71[a]	21 10
17	46 71[d]	46 66[d]	47 38	47 39	46 66	46 25	47 20	47 28
18	42 04	41 96	41 73	41 71	42 01	41 85	41 87	41 93
19	46 51[d]	46 44[d]	46 25	46 25	46 46	47 25	46 38	46 20
20	31 00	30 99	30 94	30 96	30 97	30 83	30 83	30 00
21	34 23	34.23	34 17	34 17	34 21	34 00	34 04	32.40
22	33 23[a]	33 01[a]	32 73[a]	32 79[a]	33.20	32 14	32 13	30.87[b]
23	28 80[c]	28.14[c]	28 28	28 28[b]	28 80[c]	27 89	27 90	27.97[d]
24	16.59	16 99	17 08	17 08	16 58	15 45	15 44	20.48
25	15.58	15 39	15 60	15 58	15 47	14 83	14 30	15 47
26	17 46	17 35	17 54	17 54	17 40	16 81	16 74	20 67
27	26 19	26 19	26 08	26 10	26 18	26 02	25 93	26 04[a]
28	180 23	180 20	176 31	176 31	180 18	175 93	175 86	175 88
29	33 30[a]	33 28[a]	33 20	33 22	33 28[a]	33 14	33 11	32 95
30	23 85[b]	23 76[b]	23 73[b]	23 74	23 74[b]	23 66	22 95	23 84[c]
MeCO		170 63						
MeCO		21 15						

[a–d]Assignments in any vertical column may be reversed



	R
4	Xyl
5	Gal
6	Gal^6—Ara

identical ^{13}C NMR signals (Table 2) but as both were pentoses, could not be differentiated by mass spectrometry. However, ^{13}C INEPT experiments (no decoupling) confirmed their anomeric configurations as β- and α-, consistent with xylose and arabinose, respectively [11] The inter-sugar connectivities were established by observation of the glycosidation shifts [9] as well as the acetylation shifts [12] between **4**, **5**, **6** and their respective acetylated derivatives **8**, **9**, **10** The M_rs and sequencing of the sugars in the saponins were established by fast atom bombardment spectrometry (FABMS) in the positive [13] and negative [14] ion modes It was evident that the C-28 ester bond was relatively more vulnerable to fragmentation than the C-3 glycosidic bond since in each case, ions corresponding to the totality of the sugar units at C-28 were prominent.

Compound **4** gave fragment ions at *m/z* 1183 $[M+Na]^+$, 1051 $[M+Na-Xyl]^+$, 903 $[M+Na-Xyl-Rha]^+$, 773 $[M+Na-Xyl-Rha-Xyl]^+$, 641 $[M+Na-Xyl-Rha-Xyl-Ara]^+$ and 565 $[Xyl+Rha+Xyl+Ara+Na]^+$ Hence, the terminal xylose was linked to rhamnose; arabinose was directly linked to C-28. The sugar sequencing determined by mass spectrometry was supported by partial acid hydrolysis with xylose and

Table 2 ^{13}C NMR chemical shifts of sugar moieties of compounds **4**, **6**–**10** in pyridine-d_5

C		4		6	7		8		9		10
3-*O*-Gal	1	106 93		106 95	106.95		101.33		101.49		104.89
	2	75 83		75 85	75 83		73 46		73.47		73 67
	3	78 79		78.83	78.79		72.04		72.00		78.35
	4	71.83		71 83	71.83		74 09		73 71		72 20
	5	71 26		78.09	78 06		71 26		70.67		78 04
	6	63 05		63 05	63.03		61.75		61 71		62 64
28-O-Ara	1	93 41		93.29			93 03		93 17		92 82
	2	75 42		75 45			74.71		72.97		73 56
	3	78.52		78.33			71 48		71 81		75.18
	4	68 76		70 88			69.00		69.41		72 08
	5	67 15		67 07			67.27		67.35		66.81
28-*O*-Xyl	1	105 90		105.36			98 67		98.77		101 05
	2	75 02[a]		75 45			72.91		72.46		73.56
	3	79 11		88.14			73 46		73 47		79.76
	4	65 80		68.67			64.87		62.96		68 56
	5	64.51[b]		67 07[c]			64 51[d]		62.56		66.96
28-*O*-Rha	1	100 71		100 74			103 20		103.13		103 15
	2	71.66		71 54			71 39		70 67		72.54
	3	75.75		75.26			73.13		72.97		75 18
	4	82 30		82.40			77.00		79 61		78 39
	5	69.24		69 74			68 39		69 74		69.52
	6	18 70		18.57			17 50		18.57		18 53
28-*O*-Xyl	1	105 25	Gal	105 81		Xyl	98.69	Gal	101 82		101.34
	2	75.02[a]		75.85			71 91		73.71		73.67
	3	73.38		78 39			76.26		76 99		79 76
	4	68.76		71.83			69 00		71.81		72.20
	5	62 64[b]		75.26			62 18[d]		69.58		78.29
	6	—		66.37[c]			—		68.56		68.52
28-*O*-Ara	1			104.65							100.33
	2			75 50							76.40
	3			74 34							72.97
	4			68 95							69.27
	5			62 45							62.45

[a–d]Assignments in any vertical column may be reversed.

galactose appearing (PC) during the first hour of hydrolysis. Close examination of the acetylation and glycosidation shifts [9, 12] confirmed 4-linked rhamnose, 3-linked xylose and 3-linked arabinose. The FAB mass spectrum of the peracetylated derivative of **4** (compound **8**) gave a molecular ion at *m/z* 1729 $[M+Na]^+$, corresponding to a total of 13 acetate units, giving compound **8** as 3-*O*-(2,3,4,6-tetra-*O*-acetyl)galactosyl 28-*O*-(2,3,4-tri-*O*-acetyl) xylosyl-(2,3-di-*O*-acetyl)rhamnosyl-(2,4-di-*O*-acetyl) xylosyl-(2,4-di-*O*-acetyl) arabinosylolean-12-en-28-oic acid ester

The configurations of the inter-sugar linkages (α or β) were elucidated by using ^{13}C NMR INEPT experiments to determine the $^1J_{C-H}$ coupling constants of the anomeric carbon atoms, because it has been reported [11] that the magnitude of such couplings reflects the nature of linkages; 158–162 Hz and 169–171 Hz for β- and α-linkages, respectively. Thus, compound **4** showed reasonances at δ 106.87 (C-1, 159.1 Hz), 105.20 (C-1, 162.3 Hz), 100.65 (C-1, 169.7 Hz), 105.82 (C-1, 161.2 Hz) and 93.33 (C-1, 170.9 Hz), consistent with β-galactopyranosyl, β-xylopyranosyl, α-rhamnopyranosyl, β-xylopyranosyl and α-arabinopyranosyl configurations, respectively. Hence, compound **4** was characterized as 3-*O*-β-galactopyranosyl 28-*O*-β-xylopyranosyl (1-4)-α-rhamnopyranosyl(1-3)-β-xylopyranosyl(1-3)-α-arabinopyranosylolean-12-en-28-oic acid ester.

Compound **5** gave fragment ions in the FAB mass spectrum, *m/z* 1213 $[M+Na]^+$, 1051 $[M+Na-Gal]^+$, 903 $[M+Na-Gal-Rha]^+$, 773 $[M+Na-Gal-Rha-Xyl]^+$, 641 $[M+Na-Gal-Rha-Xyl-Ara]^+$ and 595 $[Gal+Rha+Xyl+Ara+Na]^+$. The fragment at *m/z* 1051 suggested galactose as the terminal sugar which was supported by partial acid hydrolysis. It was noted that the remaining fragments were identical to compound **4**. The FAB mass spectrum of the acetylated derivative of **5** (compound **9**) gave a molecular ion at *m/z* 1801 $[M+Na]^+$ corresponding to a total of 14 acetyl units, giving compound **9** as 3-*O*-(2,3,4,6-tetra-*O*-acetyl) galactopyranosyl 28-*O*-(2,3,4,6-tetra-*O*-acetyl) galactopyranosyl-(2,3-di-*O*-acetyl)rhamnopyranosyl-(2,4-di-*O*-acetyl)xylopyranosyl-(2,4-di-*O*-acetyl) arabinopyranosylolean-12-en-28-oic acid ester. Close examination of the ^{13}C NMR spectrum of its acetate **9**, and the acetylation shifts [12] as compared to the spectra of compounds **4** and **8** revealed identical inter-sugar linkages to those in compound **4**. Deacetylation of compound **9** gave compound **5** in very low yield and as a consequence, the ^{13}C NMR spectrum

for the latter could not be reported in the present work. Analysis of the $^1J_{C-H}$ couplings of the anomeric carbons in compound **9** by ^{13}C NMR INEPT spectroscopy gave δ101.5 (C-1, 165.5 Hz), 101.84 (C-1, 166.6 Hz), 98.74 (C-1, 170 9 Hz), 103 13 (C-1, 163.3 Hz) and 93 19 (C-1, 175.2 Hz), consistent with β-galactopyranosyl, β-galactopyranosyl, α-rhamnopyranosyl, β-xylopyranosyl and α-arabinopyranosyl configurations, respectively. Hence compound **5** was characterized as 3-*O*-β-galactopyranosyl 28-*O*-β-galactopyranosyl(1-4)-α-rhamnopyranosyl (1-3)-β-xylopyranosyl(1-3)-α-arabinopyranosylolean-12-en-28-oic acid ester

Compound **6** gave FAB mass spectrum fragment ions at *m/z* 1345 $[M+Na]^+$, 903 $[M+Na-Ara-Gal-Rha]^+$, 727 $[Ara+Gal+Rha+Xyl+Ara+Na]^+$, 594 $[Ara+Gal+Rha+Xyl+Na]^+$ and 286 $[Xyl+Ara+Na]^+$ Hence, it was concluded that arabinose, galactose and rhamnose comprised the terminal sugars in some sequence, whilst arabinose was linked to C-28 followed by xylose. The overall sugar sequence (particularly amongst the first three sugars) was completed by FABMS (negative ion mode) [14], *m/z* 1321 $[M-H]^-$, 1190 $[M-H-Ara]^-$ indicating arabinose as the terminal sugar. Fragments 1027 $[M-H-Ara-Gal]^-$ and 617 $[M-H-Ara-Gal-Rha-Xyl-Ara]^-$, showed that the terminal arabinose was linked to galactose followed by rhamnose. Close examination of the acetylation and glycosidation shifts [9, 12] revealed 6-linked galactose, 4-linked rhamnose, 3-linked xylose and 3-linked arabinose. The FAB mass spectrum of the peracetylated derivative of **6** (compound **10**) gave FAB mass spectrum molecular ions at *m/z* 2017 $[M+Na]^+$ which suggested the incorporation of 16 acetyl groups giving compound **10** as 3-*O*-(2,3,4,6-tetra-*O*-acetyl)galactosyl 28-*O*-(2,3,4-tri-*O*-acetyl) arabinosyl-(2,3,4-tri-*O*-acetyl) galactosyl (2,3-di-*O*-acetyl)rhamnosyl-(2,4-di-*O*-acetyl) xylosyl-(2,4-di-*O*-acetyl)arabinosylolean-12-en-28-oic acid ester. Analysis of the $^1J_{C-H}$ couplings of the anomeric carbons in compound **6** by ^{13}C NMR INEPT spectroscopy gave δ106 95 (C-1, 158.0 Hz), 93.29 (C-1, 171.9 Hz), 105 36 (C-1, 163 3 Hz), 100 71 (C-1, 170 8 Hz), 105.81 (C-1, 160.1 Hz) and 104.65 (C-1, 176 2 Hz), consistent with β-galactopyranosyl, α-arabinopyranosyl, β-xylopyranosyl, α-rhamnopyranosyl and α-arabinopyranosyl configurations, respectively. Hence compound **6** was characterized as 3-*O*-β-galactopyranosyl 28-*O*-α-arabinopyranosyl(1-6)-β-galactopyranosyl(1-4)-α-rhamnopyranosyl (1-3)-β-xylopyranosyl(1-3)-α-arabinopyranosylolean-12-en-28-oic acid ester

Peracetylation was preferred to permethylation for determining the inter-sugar linkages because the parent glycosides can be recovered more easily for further analysis and subsequent biological tests Because neither compound **1** nor **2** was detected in the unhydrolysed extract of the pulp, the occurrence of **2** in the intact saponins must necessitate further investigations.

EXPERIMENTAL

Mps uncorr. IR. nujol, unless otherwise stated ^1H NMR (270 MHz) and ^{13}C NMR (67 5 MHz) in pyridine-d_5 with TMS as int standard; chemical shifts were recorded in ppm. ^{13}C NMR spectra were used to determine carbon atom type by employing a combination of broad band decoupled and DEPT 90 and/or 135 experiments FABMS was obtained with both positive and negative ion modes (glycerol) TLC of saponins was carried out on Kieselgel 60 GF254 (Merck), zones were detected with I_2 vapour and 10% H_2SO_4 at 110° PC of sugars were run on Whatman No 1 (ascending model) and detected with aniline phthalate spray at 110° The following solvent systems were used. (A) hexane–EtOAc (4 1), (B) $CHCl_3$–MeOH–H_2O (13 7 2, lower phase), (C) hexane–EtOAc (2 3) and (D) *n*-BuOH–toluene–pyridine–H_2O (5 1 3 3) Analytical GC of the TMSi-derivatives (of the sugars or the dried acid hydrolysate of the saponin plus 200 μl BSTFA with 1% TMCS and 100 μl pyridine at 80° for 2 hr) was performed on a glass column (2 m × 4 mm i d) packed with 2% SE-30, N_2 at 40 ml min^{-1}, isothermal at 190°, inj 200° and FID det 300°

Plant material The fruit of *Lagenaria breviflora* Robert (Cucurbitaceae) was collected from Ile-Ife, Nigeria and authenticated with the herbarium specimen, kept at the Forest Research Institute of Nigeria, Ibadan The fresh fruit was separated into epicarp, mesocarp (pulp) and seed The pulp was dried at 65°, powdered and kept until ready for extraction

Direct acid hydrolysis Dried powdered pulp of *L breviflora* fruit (0 1 kg) was refluxed in aq 2 M HCl for 7 hr The marc, following filtration, was extracted with 500 ml $CHCl_3$, the solvent was removed *in vacuo* and by prep TLC (system A), compounds **1** (6 5 mg) and **2** (4 1 mg) were obtained

Compound **1** Powder, identified as oleanolic acid· R_f 0 23 (solvent A), mp 295–300° IR ν_{max} cm^{-1} 3400 (OH), 1680 (COOH), 1620 (unsatn), 1220, 1230 ^1H NMR δ1.76, 1 67, 1 48, 1 35 1 26, 1 03, 0 97 (s, 7 × Me), 5 52 (H-12), ^{13}C NMR (Table 1). EIMS (for $C_{30}H_{48}O_3$) *m/z* (rel int) 456 $[M]^+$ (1 2), 249 (18.8), 248 (100), 203 (88 7), were comparable to the retro-Diels–Alder fragments of authentic oleanolic acid and literature data [7]

Compound **2** Pinkish-brown powder, identified as 3-*O*-acetyloleanolic acid R_f 0 38 (solvent A), mp 240–243°, IR ν_{max} cm^{-1} 3200 (OH of carboxyl), 1720 (vs, Ac), 1680 (COOH), 1620 (unsaturation), 1380, 1330, 1300, 1260, 1160, 1010, 980, 900 ^1H NMR δ1.77, 1 67, 1 47, 1 43, 1 29, 1 02, 0 97 (s, 7 × Me), 5 49 (H-12), ^{13}C NMR (Table 1) EIMS for $C_{32}H_{50}O_4$, *m/z* (rel. int.) 498 $[M]^+$ (0.2), 483 $[M-Me]^+$ (0 1), 455 $[M-Ac]^+$ (0 1), 439 $[M-Me-COO]^+$ (3 1), 423 (1.8), 410 (0 1), 249 (20), 248 (100), 203 (77 3), were comparable to lit data [6] and to the data obtained from the acetylated (Ac_2O in pyridine) sample of compound **1**

Extraction and isolation of saponins Powdered pulp (0 12 kg) was defatted with petrol and successively extracted with EtOAc and MeOH The MeOH extract was concd to half vol at red pres and the insol gummy ppt discarded After evapn of the solvent, the residue was dissolved in H_2O and successively partitioned with Et_2O and *n*-BuOH (satd with H_2O) The *n*-BuOH fraction was evapd to afford a crude saponin mixture from which an impure compound **3** (mixture of compounds **4** and **5**) and compound **6** were isolated by repeated prep TLC on 1 0 mm silica gel layers (solvent B) and bands recovered into MeOH.

Purification of compound **3** A dull white powder, R_f 0 38 (solvent B) mp 219–225°, IR ν_{max} cm^{-1} 3375 (OH), 1720–1540 (ester and unsatn), 1310, 1260, 1160, 1080, 1060, 1040, FABMS (positive ion mode), *m/z* 1183 $(M_1+Na]^+$ and 1213 $[M_2+Na]^+$, 1051 $[M_1+Na-132]^+$ or $[M_2+Na-162]^+$ and 641 $[M_1+Na-132-146-132-132]^+$ or $[M_2+Na-162-146-132-132]^+$ Compound **3** was peracetylated to give two clearly separated bands on prep TLC as before (R_f 0 67 and 0 30) in solvent C, which on mild alkaline hydrolysis afforded the parent saponins **4** and **5**, respectively

Compound **4** A white amorphous powder R_f 0 38 (solvent B), mp 260–266°, IR ν_{max} cm^{-1} 3350 (OH), 1730–1680 (ester CO), 1620 (unsaturation), 1260, 1060, 1040, ^{13}C NMR decoupled

spectrum (Table 1), ^{13}C NMR INEPT (no decoupling) C-1, ppm ($^1J_{C-H}$): 106.87 (159.1 Hz), 105 20 Hz (162.3 Hz); 100.65 (169.7 Hz), 105.82 (161 2 Hz) and 93 33 (170.9 Hz) FABMS (positive ion mode), *m/z* (rel. int)· 1183 $[M+Na]^+$ (65.4), 1051 $[M+Na-132]^+$ (15), 903 $[M+Na-132-146]^+$ (8 7), 773 $[M+Na-132-146-132]^+$ (10 8), 641 $[M+Na-132-146-132-132]^+$ (15 4), 565 $[M+Na-618]^+$ (57 7), 437 $[M+Na-162-132-146-132-132]^+$ (10 8), 145 (100), negative ion mode: 1159 $[M-H]^-$, 1027 $[M-H-132]^-$, 617 $[M-H-132-146-132-132]^-$, 455 $[M-H-132-146-132-132-162]^-$, 437 $[M-H-132-146-132-132-18]^-$, calculated for $C_{57}H_{92}O_{24}$, M_r 1160.

Compound **5**. A white amorphous powder, R_f 0 38 (solvent B), IR ν_{max} cm^{-1}: 3350 (OH), 1725–1680 (ester CO), 1620 (unsatn), 1060, 1040, 980, 920. ^{13}C NMR INEPT (no decoupling), C-1, ppm; ($^1J_{C-H}$). 101 5 (165.5 Hz), 103.13 (163 3 Hz); 98 74 (170 9 Hz), 93 19 (175 2 Hz) and 101 84 (166.6 Hz). FABMS (positive ion mode), *m/z* (rel. int.) 1213 $[M+Na]^+$ (35.5), 1051 $[M+Na-162]^+$ (15), 903 $[M+Na-162-146]^+$ (8 3), 773 $[M+Na-162-146-132]^+$ (10 8), 641 $[M+Na-162-146-132-132]^+$ (15), 595 $[M+Na-618]^+$ (26.9), 437 $[M+Na-162-146-132-132-162-18]^+$ (8), negative ion mode: 1189 $[M-H]^-$, 1027 $[M-H-162]^-$, 617 $[M-H-162-146-132-132]^-$, 455 $[M-H-162-146-132-132-162]^-$, 437 $[M-H-162-146-132-132-162-18]^-$, calculated for $C_{58}H_{94}O_{25}$, M_r 1190.

Compound **6**. A white powder, R_f 0.32 (solvent B); mp 230–232°, IR ν_{max} cm^{-1}: 3350 (OH), 1760–1680 (ester CO), 1620 (unsatn), 1060, 1020, 960 ^{1}H NMR δ1.66, 16 3, 1.45, 1.32, 1.30, 1 02, 0 94 (*s*, 7 × Me), 5.50 (H-12), 4.29 (1H, *d*, J = 4 4 Hz, anomeric proton of Ara), 1 75 (3H, *d*, J = 4 9 Hz, H-6 of Rha), 4.52 (1H, *d*, J = 6.9 Hz, anomeric proton of 28-*O*-Ara), 4 6 (1H, J = 8 1 Hz, anomeric proton of term Gal), 4.97 (1H, *d*, J = 7.7 Hz, anomeric proton of Xyl), 5.23 (1H, *d*, J = 3.7 Hz, anomeric proton of Gal), 5.7 (1H, *d*, J = 3 7 Hz, anomeric proton of Rha) ^{13}C NMR INEPT, C-1, ppm, ($^1J_{C-H}$): 106 95 (158 Hz), 105.81 (160.1 Hz); 105 36 (163.3 Hz), 104.65 (176.2 Hz), 100.71 (170 8 Hz) and 93.29 (171 9 Hz) ^{13}C NMR (Table 1). FABMS (positive ion mode), *m/z* (rel. int)· 1345 $[M+Na]^+$ (48), 903 $[M+Na-132-162-146]^+$ (8 3), 727 $[M+Na-618]^+$ (20), 594 $[M+Na-618-132]^+$ (6.3), 286 $[M+Na-618-132-162-146]^+$ (100), 437 $[M+Na-132-162-146-132-132-162-18]^+$ (8.7); (negative ion mode), *m/z* 1321 $[M-H]^-$, 1190 $[M-H-132]^-$, 1027 $[M-H-132-162]^-$, 617 $[M-H-132-162-146-132-132]^-$, calculated for $C_{63}H_{102}O_{29}$, M_r 1322.

Compound **7** A brown solid, obtained by alkaline hydrolysis of compounds **4–6**, R_f 0 87 (solvent B); IR ν_{max} cm^{-1} 3425 (OH), 1720 (COOH), 1620 (unsatn), 1040, 920. ^{13}C NMR decoupled spectrum (Table 1) FABMS (positive ion mode) *m/z*, (rel. int.)· 641 $[M+Na]^+$ (100), 493 $[M+Na-Me]^+$ (18), 477 $[M+Na-162]^+$ (28), 459 $[M+Na-162-18]^+$ (87), calculated for $C_{36}H_{38}O_8$, M_r 618 identified as 3-*O*-β-galactopyranosyloolean-12-en-28-oic acid.

Acid hydrolysis Compounds **4–6**, individually refluxed in 2 M HCl for 5 hr gave oleanolic acid, identical to compound 1 (co-TLC, co-IR, ^{13}C NMR, EIMS) PC (solvent D) of the hydrolysates showed similar sugars R_t 0.28 (Gal), 0 33 (Ara), 0 43 (Xyl) and 0 51 (Rha); GC of TMSi-derivatives, R_t, min: 13.3, 16 5 (Gal), 5.0, 6.3 (Ara), 5.3, 7.5 (Xyl), and 5 3, 7.0 (Rha). Mild acid hydrolysis was carried out with 1 M HCO_2H and was similarly examined for sugars every 30 min.

Alkaline hydrolysis Compounds **4–6**, individually refluxed in 2 M NaOH for 3 hr, neutralized and partitioned with *n*-BuOH (satd with H_2O) Prep. TLC of the organic layer gave compound **7** (TLC, FABMS, IR) while PC (solvent D) of the hydrolysate (after further acid hydrolysis) gave similar sugars as above. Mild alkaline hydrolysis of the acetylated derivatives was performed at room temp. for 24 hr and the appropriate *n*-BuOH layer was evapd to recover the parent saponins. Compound **2** was similarly deacetylated.

Peracetylation. Compounds **1**, **4**, **5** and **6**, were individually treated with Ac_2O in pyridine at room temp. for 48 hr and purified by prep. TLC (solvent C) to give compounds **2**, **8**, **9** and **10**, respectively.

Compound **8**. Brown solid R_f 0.67 (solvent C); IR ν_{max} cm^{-1}· 1740 (−Ac), 1620 (unsatn), 1450, 1370, 1040, 980. FABMS (positive ion mode), *m/z* 1729 $[M+Na]^+$ for $C_{83}H_{118}O_{38}$, M_r 1708.

Compound **9**. Brown solid, R_f 0.30 (solvent C), IR ν_{max} cm^{-1} 1740 (−Ac), 1620 (unsatn), 1420, 1370, 940. FABMS (positive ion mode), *m/z* 1801 $[M+Na]^+$ for $C_{85}H_{120}O_{38}$, M_r 1778

Compound **10**. Dark semi-solid, R_f 0.69 (solvent C), IR-ν_{max} cm^{-1} 1740 (−Ac), 1620 (unsatn), 1560, 1430, 1370, 1040, 940; FABMS (positive ion mode), *m/z* 2017 $[M+Na]^+$ for $C_{95}H_{124}O_{45}$, M_r 1994

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