

75. New Triterpenoid *N*-Acetylglycosides with Molluscicidal Activity from *Tetrapleura tetraptera* TAUB.

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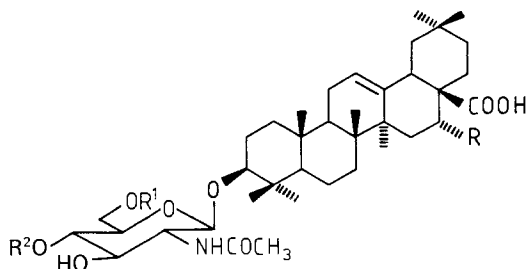
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Activity-guided fractionation of the MeOH extract of the fruits of *Tetrapleura tetraptera* TAUB. (Mimosaceae) afforded 4 saponins **1–4**, which exhibited strong molluscicidal properties against the schistosomiasis-transmitting snails *Biomphalaria glabrata*. Chemical, enzymatic, and spectral methods (DCI-MS, ¹H-NMR, ¹³C-NMR) showed that they were *N*-acetylglycosides of oleanolic acid and of echinocystic acid. Apart from saponin **1** (aridanin), previously isolated from this plant, glycosides **2–4** are new naturally occurring compounds.

Introduction. – There is still an urgent need for highly potent plant molluscicides in order to avoid the transmission of the parasitic disease schistosomiasis [1]. *Tetrapleura tetraptera* TAUB. (Mimosaceae), locally known as Aridan, is a large tree growing throughout the rain forest belt of West Africa. The plant has many traditional uses, mainly in the management of convulsions, leprosy, inflammation, and rheumatic pains [2]. Molluscicidal activity of this plant was reported by Adewunmi and Marquis [3]. The activity seemed to be linked to triterpenoid saponins and coumarinic compounds. Because of the strong molluscicidal properties of the fruits of *Tetrapleura tetraptera*, field trials have been carried out, and this tree is now considered to be a promising plant in the local control of schistosomiasis [4]. Only one active compound, a mono-*N*-acetylglycoside of oleanolic acid (= 3 β -hydroxyolean-12-en-28-oic acid), aridanin (**1**), has been isolated and identified from this species [5]. Thus, further phytochemical investigation is required. The present paper deals with the identification of additional saponins from the fruits of *Tetrapleura tetraptera*.

Results. – Dried fruit pulp of *Tetrapleura tetraptera* (200 g) collected in Nigeria was extracted successively with CH₂Cl₂, MeOH, and H₂O. The MeOH extract (80 g) which is molluscicidal at 25 ppm was then partitioned between H₂O and BuOH. Fractionation of 20 g of the BuOH extract by column chromatography on silica gel was monitored by TLC. Several of the 17 fractions collected presented a strong molluscicidal activity (< 20 ppm), namely *Fractions 7, 9, 10, 11, and 12* (1150, 500, 450, 500, and 1100 mg, resp.). These fractions were further separated by reversed-phase chromatography on *RP-8* or liquid-liquid chromatography [6] (see *Exper. Part*) to afford four saponins **1–4**. Compound **1** (995 mg) was obtained from *Fraction 7*, **2** (60 mg) from *Fraction 9*, **3** (220 mg) from *Fractions 11 and 12*, and **4** (90 mg) from *Fraction 12*.

Acidic hydrolysis with 4*N* HCl of saponins **1, 3, and 4** afforded oleanolic acid, identified by comparison with an authentic sample (TLC, HPLC). The aglycone of



- 1** R = R¹ = R² = H, Aridanin
- 2** R = α -OH, R¹ = R² = H
- 3** R = R¹ = H, R² = Gal
- 4** R = H, R¹ = Glc, R² = H

saponin **2** could not be obtained under the same conditions as decomposition occurred. Attempts with different kinds of enzymatic hydrolysis using galactosidase, glucosidase, and glucuronidase failed. Finally, acidic hydrolysis with 2N H₂SO₄ yielded an aglycone with lower *R_f* value on silica-gel TLC than oleanolic acid (see below). TLC analysis of the four saponin hydrolysates showed the presence of one ninhydrin-positive sugar in all cases, together with galactose for saponin **3** and glucose for saponin **4**. This sugar was identified as glucosamine hydrochloride [7] by comparison on cellulose TLC and silica-gel TLC with an authentic sample. DCI-MS (NH₃, positive-ion mode) and NMR spectra of the saponins **1–4** confirmed the presence of the 2-acetamido-2-deoxy- β -D-glucopyranose moiety and established the structure of **1** as 3-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)oxy]olean-12-en-28-oic acid, a compound previously isolated from *Pithecellobium sp.* (Leguminosae) [8] and from the fruit of *Tetrapleura tetraptera* [5]. The compound was named aridanin, and the spectral data of **1** are in accordance with those reported [5] [8].

The presence of a N-containing monosaccharide in all four saponins **1–4** was confirmed by DCI-MS which showed quasimolecular ion peaks $[M + H]^+$ at *m/z* 660, 676, 822, and 822, thus indicating molecular weights of 659, 675, 821, and 821, respectively. Furthermore, a typical NH signal at *ca.* 9.0 ppm (*d*, *J* = 9, 1 H) in the ¹H-NMR spectra of **1–4** was observed. The presence in all ¹³C-NMR spectra of a CH₃ signal at 23.7 ppm, a C=O signal at *ca.* 170 ppm, and a characteristic signal at 58 ppm due to C(2') of an acetylated *N*-glucosamine indicated clearly the presence of the 2-acetamido-2-deoxy- β -D-glucopyranose moiety. The β -D-configuration was confirmed by ¹H-NMR data (H–C(1') at 5.0 ppm (*J* = 8 Hz)).

The aglycone of **2** could be identified as echinocystic acid (= 3 β ,16 α -dihydroxyolean-12-en-28-oic acid) by comparison with an authentic sample showing the same EI-MS fragmentation pattern and HPLC retention time and co-migration on TLC.

The DCI-MS and NMR spectra of **2** showed that it is 3-[(2-acetamido-2-deoxy- β -D-glucopyranosyl)oxy]-16 α -hydroxyolean-12-en-28-oic acid. This saponin has previously been reported as a prosapogenin obtained after partial hydrolysis of Entada saponin III, a product isolated from the bark of *Entada phaseolides* (Leguminosae). All the spectroscopic data are in good agreement with published values [10].

The DCI-MS of **2** showed a quasimolecular ion at *m/z* 676 ($[M + H]^+$), 16 amu above the one of **1**. Since the sugar moieties are identical in **1** and **2**, the aglycone of **2** must have an additional O-atom. In fact, EI-MS of the product obtained after acidic hydrolysis of **2** showed clearly a molecular peak at *m/z* 472 ($[M]^+$) and a typical *retro-Diels-Alder* fragmentation with peaks at *m/z* 264 ($[M - C_{14}H_{24}O]^+$), 246 ($[(264 - H_2O)^+]$), and 208

([C₁₄H₂₄O]⁺), indicating the presence of an OH group at ring C, D, or E of the triterpene. Chemical shifts of the triterpenoid moiety in the ¹³C-NMR spectrum of **2** were in good agreement with a 16 α -OH substitution pattern [9].

In addition, the ¹³C-NMR spectrum of **2** was very similar to that of commercially available echinocystic acid 3-*O*-glucoside; the signal at 89.0 ppm indicated the substitution at C(3) of the terpenoid moiety.

Enzymatic hydrolysis of saponin **3** with galactosidase afforded a prosapogenin (*m/z* 660 [*M* + H]⁺) identified as aridanin (**1**; ¹H-NMR, ¹³C-NMR). Thus, galactose is the terminal sugar. The interglycosidic linkage was determined by comparison of the ¹H-NMR spectra (CDCl₃) of the peracetylated derivatives **1a** and **3a** of saponins **1** and **3**, respectively, and by the ¹³C-NMR spectrum of **3**.

Thus, the structure of **3** is established as 3- $\{[O\text{-}\beta\text{-D-galactopyranosyl-(1}\rightarrow\text{4)-(2-acet-amido-2-deoxy-}\beta\text{-D-glucopyranosyl)]oxy\}$ olean-12-en-28-oic acid which is a new natural product.

The DCI-MS of **3** exhibited quasimolecular ions at *m/z* 839 ([*M* + NH₄]⁺) and 822 ([*M* + H]⁺). A peak at *m/z* 660 ([(*M* + H) – 162]⁺), resulting from the elimination of a hexosyl moiety, could be observed.

Table 1. ¹H-NMR Chemical Shifts of the Glycosidic Moieties of Peracetates **1a** and **3a**^{a)}

Sugar moiety ^{b)}		1a		3a	
Peracetylated GlcNAc	H–C(1')	4.72	<i>d</i> , <i>J</i> = 8	4.46	<i>d</i> , <i>J</i> = 8.1
	H–C(2')	3.77	<i>ddd</i> , <i>J</i> = 8, 9, 9.5	4.00	<i>m</i> \approx <i>ddd</i>
	H–C(3')	5.32	<i>dd</i> , <i>J</i> = 9.5, 9.5	5.10	<i>dd</i> , <i>J</i> = 9, 9
	H–C(4')	5.00	<i>dd</i> , <i>J</i> = 9.5, 9.5	3.72	<i>dd</i> , <i>J</i> = 9, 9
	H–C(5')	3.6–3.7	<i>m</i>	3.59	<i>m</i>
	H–C(6')	4.06	<i>dd</i> , <i>J</i> = 12, 2	4.0–4.20	unres.
	H'–C(6')	4.24	<i>dd</i> , <i>J</i> = 12, 2	4.4–4.50	unres.
	NH–C(2')	6.00	<i>d</i> , <i>J</i> = 9	5.60	<i>d</i> , <i>J</i> = 9
Peracetylated Gal	H–C(1'')			4.50	<i>d</i> , <i>J</i> = 8
	H–C(2'')			5.12	<i>dd</i> , <i>J</i> = 8, 10
	H–C(3'')			4.96	<i>dd</i> , <i>J</i> = 10, 3
	H–C(4'')			5.35	<i>dd</i> , <i>J</i> = 3, < 1
	H–C(5'')			3.87	<i>dd</i> , <i>J</i> = 7, < 1
	H–C(6'') } H'–C(6'')			4.0–4.20	unres.

a) Spectra were measured in CDCl₃.

b) GlcNAc = 2-acetamido-2-deoxy- β -D-glucopyranosyl, Gal = β -D-galactopyranosyl.

In the peracetates **1a** and **3a**, the ¹H-NMR signals of the sugar moieties appeared in the region 3.5–5.5 ppm, and the use of homonuclear COSY experiment allowed the attribution of all these protons (see Table 1). The signal at 5.00 ppm for H–C(4') of peracetate **1a** was shifted upfield to 3.72 ppm in the ¹H-NMR spectrum of **3a**, in accordance with a 4'-*O* substitution. Indeed, osidic protons appear at two different ranges of chemical shifts, from 3.0 to 4.5 ppm for CH₂OAc, CH₂OR, and CHOR and from 4.5 to 5.5 for CHOAc [11]. Further evidence for the 4'-*O* substitution of the inner *N*-acetyl- β -D-glucosamine moiety was observed in the ¹³C-NMR of **3**. The C(4') signal was shifted downfield by 11.0 ppm to 83.5 ppm, the C(3') and C(5') signals being shifted upfield by 2.5 and 1.9 ppm, respectively, whereas the other C-atoms remained almost unaffected, as compared to **1** (see Table 2). These results are in good accordance with the glycosylation rules established by Konishi *et al.* [12].

The β -conformation of the galactose moiety was deduced from the ¹H-NMR spectrum, where the anomeric proton appeared as *d* at 5.00 ppm (*J* = 8.5, H–C(1'')).

The DCI-MS of **4** (*m/z* 822 ([*M* + H]⁺), 660 (cleavage of a hexosyl moiety)) indicated that the glucosyl moiety was in the terminal position, and the interglycosidic linkage was determined by means of ¹³C-NMR spectroscopy (downfield shift of C(6') by 7.4 ppm as

Table 2. ^{13}C -NMR Data of 1–4^{a)}

	1	2	3	4
C(1)	38.5 (<i>t</i>)	38.5	38.5	38.5
C(2)	26.2 (<i>t</i>)	26.3	26.3	26.5
C(3)	89.2 (<i>d</i>)	89.0	89.3	89.1
C(4)	39.2 (<i>s</i>)	39.2	39.2	39.2
C(5)	55.7 (<i>d</i>)	55.7	55.7	55.6
C(6)	18.5 (<i>t</i>)	18.5	18.5	18.4
C(7)	33.2 (<i>t</i>)	33.4	33.3	33.1
C(8)	39.7 (<i>s</i>)	39.8	39.7	39.6
C(9)	48.0 (<i>d</i>)	47.1	48.0	47.9
C(10)	36.9 (<i>s</i>)	36.9	36.9	36.9
C(11)	23.6 (<i>t</i>)	23.7	23.7	23.7
C(12)	122.5 (<i>d</i>)	122.1	122.4	122.6
C(13)	144.8 (<i>s</i>)	145.2	144.9	144.6
C(14)	42.0 (<i>s</i>)	42.0	42.0	41.8
C(15)	28.3 (<i>t</i>)	36.1	28.3	28.2
C(16)	23.7 (<i>t</i>)	74.8 (<i>d</i>)	23.7	23.7
C(17)	46.7 (<i>s</i>)	49.0	46.7	46.5
C(18)	42.1 (<i>d</i>)	41.5	42.1	42.0
C(19)	46.5 (<i>t</i>)	47.3	46.5	46.6
C(20)	30.9 (<i>s</i>)	31.0	30.9	30.9
C(21)	34.1 (<i>t</i>)	36.1	34.2	34.1
C(22)	33.2 (<i>t</i>)	32.5	33.2	33.1
C(23)	28.3 (<i>q</i>)	28.0	28.0	28.0
C(24)	16.9 (<i>q</i>)	16.9	16.9	16.9
C(25)	15.4 (<i>q</i>)	15.5	15.3	15.3
C(26)	17.3 (<i>q</i>)	17.5	17.4	17.3
C(27)	26.1 (<i>q</i>)	27.2	26.1	26.1
C(28)	180.3 (<i>s</i>)	180.0	180.5	180.2
C(29)	33.2 (<i>q</i>)	33.4	33.2	33.3
C(30)	23.7 (<i>q</i>)	24.9	23.7	23.7
C(1')	104.5 (<i>d</i>)	104.8	104.4	104.9
C(2')	58.0 (<i>d</i>)	57.8	57.4	57.7
C(3')	76.1 (<i>d</i>)	76.2	73.6	75.9
C(4')	72.5 (<i>d</i>)	72.5	83.5	72.5
C(5')	78.1 (<i>d</i>)	78.2	76.2	76.9
C(6')	62.9 (<i>t</i>)	62.8	62.5	70.3
COCH ₃	170.2 (<i>s</i>)	170.2	169.9	170.1
COCH ₃	23.7 (<i>q</i>)	23.7	23.7	23.7
C(1'')			105.9	105.3
C(2'')			72.5	75.2
C(3'')			75.1	78.4
C(4'')			70.0	71.6
C(5'')			77.2	78.4
C(6'')			61.9	62.7

^{a)} Spectra were measured in (D₅)pyridine.

compared to **1**, see Table 2). Thus, **4** is 3- $\{[O\text{-}\beta\text{-D-glucopyranosyl-(1}\rightarrow\text{6)-(2-acetamido-2-deoxy-}\beta\text{-D-glucopyranosyl)]oxy\}$ olean-12-en-28-oic acid, a new glucoside of aridanin.

Discussion. – In spite of several reports on the molluscicidal activity of *Tetrapleura tetraptera* and aridanin [13–17], this is the first time that other active saponins have been

isolated from this plant. The three isolated saponins **2–4** kill *Biomphalaria glabrata* snails within 24 h at concentrations of 20, 2.5, and 5 ppm, respectively, and were still active within 48 h at concentrations of 10, 1.25, and 2.5 ppm. In the same bioassay, aridanin (**1**) was active only at a concentration of 20 ppm. It is interesting to notice that glycosylation of **1** increases the toxicity of the saponins to *Biomphalaria glabrata* snails.

Saponins from *Tetrapleura tetraptera* are among the most powerful natural molluscicides and have similar potencies to those isolated from *Phytolacca dodecandra* [18] and *Swartzia madagascariensis* [19], two plants with potential for use in the local control of schistosomiasis. In view of these activities, further phytochemical investigation of the complex saponin extract of *Tetrapleura tetraptera* is underway in order to discover other highly potent molluscicidal compounds.

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Experimental Part

General. Echinocystic acid from Carl Roth, Karlsruhe, echinocystic acid 3-*O*-glucoside from Sarsyntex, Bordeaux, and *N*-acetyl- β -*D*-glucosamine from Eastman Kodak Co., Rochester. Normal-phase column chromatography: silica gel 60 (40–63 μ m, Merck, Darmstadt). Reversed-phase chromatography: Büchi B 681 MPLC system with a 2.6 \times 46-cm column packed with RP-8 material (15–25 μ m, Merck No. 9324). TLC: silica-gel-pre-coated Al sheets (Merck) with AcOEt/MeOH/H₂O 17:2:1 (*sys*t. I) and petroleum ether/AcOEt 1:1 (*sys*t. II), pre-coated RP-8 nanoplates (Merck) with MeOH/H₂O 7:3, detection with Godin reagent [20], or cellulose-pre-coated Al sheets (Merck) with AcOEt/pyridine/AcOH/H₂O 5:5:1:3 (*sys*t. III). Anal. HPLC: Nucleosil 120-5 C-8 column (Macherey-Nagel), Spectra-Physics-8700 pump, detection at 206 nm with a LKB-2151 detector. Liquid-liquid chromatography: DCCC-Büchi-670 chromatograph equipped with 294 tubes (40 cm, i.d. 2.7 mm). Final purification of saponins was achieved on a 2.5 \times 45 cm Sephadex-LH-20 column (Pharmacia) with MeOH or CH₂Cl₂/MeOH 1:2. M.p.: Mettler FP 80/82 hot stage apparatus; uncorrected. IR spectra: Philips PU 9700 spectrophotometer; in KBr. ¹H-NMR and ¹³C-NMR spectra: Varian VXR-200 equipped with switchable 5-mm probe at 200 and 50.1 MHz, resp., (D₂)pyridine solns. for saponins **1–4** (30 mg/0.8 ml), and CDCl₃ solns. for peracetates **1a** and **3a** (15 mg/0.8 ml); chemical shifts in δ values (ppm) relative to TMS as internal standard; multiplicities determined by DEPT experiments. EI- and DCI-MS (NH₃, positive-ion mode): Nermag RI030 spectrometer. Molluscicidal tests were carried out as previously described in [21].

Extraction and Isolation. The dried pulp of the fruit (200 g), collected near Ile-Ife, Nigeria, was extracted at r.t. successively with CH₂Cl₂ (3 \times 500 ml), MeOH (3 \times 500 ml), and H₂O (3 \times 500 ml). The MeOH extract (80 g) was partitioned between H₂O (500 ml) and BuOH (500 ml) and the BuOH extract (20 g) separated on a silica-gel column with CHCl₃/MeOH/H₂O 85:11:1 \rightarrow 70:30:5 \rightarrow 50:50:0 (monitoring by TLC) to afford 17 fractions. Purification of Fraction 7 (1150 mg) by filtration on a Sephadex-LH-20 column with CH₂Cl₂/MeOH 1:2 afforded **1** (995 mg). Reversed-phase chromatography of Fractions 9 (500 mg) and 11 (500 mg) by medium-pressure liquid-chromatography (MPLC) on RP-8 using MeOH/H₂O 7:3 afforded **2** (60 mg) and **3** (170 mg). Droplet counter-current chromatography (DCCC) of Fraction 12 (1000 mg) with CHCl₃/MeOH/H₂O 7:13:8 (ascending mode) yielded **3** (50 mg) and **4** (90 mg).

Acidic Hydrolysis. Saponins **1**, **3**, and **4** (2 mg) were refluxed with 4*N* HCl (2 ml) for 2 h. The mixtures were then extracted with AcOEt. The org. layers were checked by TLC (silica gel, *sys*t. II), HPLC (RP-8, CH₃CN/H₂O, 65:35), and EI-MS for aglycones. The aq. phases were neutralized with NaHCO₃ and the sugars extracted with pyridine and analyzed by TLC on silica gel with AcOEt/MeOH/H₂O/AcOH 65:15:15:20 (detection with *p*-anisidine phthalate) and on cellulose with *sys*t. III (detection with ninhydrin). Saponin **2** (2 mg) was refluxed with 2*N* H₂SO₄ (2 ml) for 1 h, and then the mixture was treated as above.

Enzymatic Hydrolysis. Saponin **3** (40 mg) and β -*D*-galactosidase (40 mg) from *Helix pomatia* (G-7138, Sigma, St-Louis) were dissolved in acetate buffer, pH 5.5 (40 ml). The mixture was kept at 37° and the reaction periodically checked by TLC (silica gel, *sys*t. I). After 7 days, the mixture was extracted with 2 \times 40 ml of BuOH and the org.

residue purified by silica-gel column chromatography using $\text{CHCl}_3/\text{MeOH}$ 95:5: 15 mg of aridanin (**1**); TLC, HPLC, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$.

Acetylation. Compounds **1** and **3** (15 mg each) were dissolved in $\text{Ac}_2\text{O}/\text{pyridine}$ 1:1 and stirred at r.t. for 48 h. The mixtures were then poured into ice/ H_2O and the precipitates purified on a *Sephadex-LH-20* column ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 1:2) to afford peracetates **1a** (15 mg) and **3a** (17 mg), resp.

Aridanin (= 3-[2-Acetamido-2-deoxy- β -D-glucopyranosyl]oxy]olean-12-en-28-oic Acid; **1**). White amorphous powder. M.p. 270–275° (dec.). IR: 3400–3200 (OH), 2920, 1650, 1540 (CONH). $^1\text{H-NMR}$ (200 MHz): 0.78, 0.97, 1.00, 1.02, 1.21, 1.31 (21 H, 7 CH_3); 2.17 (NHCOCH₃); 3.8–5.10 (carbohydrate protons); 5.00 (*d*, *J* = 8, H–C(1')); 5.5 (br. *t*, H–C(12)); 8.94 (*d*, *J* = 9, NH). $^{13}\text{C-NMR}$ (50.1 MHz): Table 2. DCI-MS: 660 [(*M* + H)⁺], 457 [(*M* + H) – 203]⁺, 204 [(203 + H)⁺].

Acid hydrolysis of **1** afforded oleanolic acid and β -D-glucosamine·HCl.

Peracetyl Derivative 1a. White amorphous powder. M.p. 258–260° (dec.). $^1\text{H-NMR}$ (200 MHz; see also Table 1): 0.72, 0.73, 0.89, 0.91, 1.10, 1.24, (21 H, 7 CH_3); 1.91, 2.00, 2.02, 2.05 (4 COCH₃); 3.8–5.50 (carbohydrate protons); 4.72 (*d*, *J* = 8, H–C(1')); 5.25 (br. *t*, H–C(12)); 5.98 (*d*, *J* = 9, NH). DCI-MS: 803 [(*M* + NH₄)⁺], 786 [(*M* + H)⁺].

3-[2-Acetamido-2-deoxy- β -D-glucopyranosyl]oxy]-16 α -hydroxyolean-12-en-28-oic Acid (**2**). White amorphous powder. M.p. 265–270° (dec.). IR: 3400–3200 (OH), 2900, 1650, 1535 (CONH). $^1\text{H-NMR}$ (200 MHz): 0.82, 0.99, 1.02, 1.07, 1.19, 1.83 (21 H, 7 CH_3); 2.15 (NHCOCH₃); 3.8–5.10 (carbohydrate protons); 5.05 (*d*, *J* = 8.2, H–C(1')); 5.23 (br. *t*, H–C(12)); 8.95 (*d*, *J* = 9, NH). $^{13}\text{C-NMR}$ (50.1 MHz): Table 2. DCI-MS: 676 [(*M* + H)⁺], 490 [(472 + NH₄)⁺], 455 [(472 + H) – 18]⁺, 204 [(203 + H)⁺].

Acidic hydrolysis of **2** afforded β -D-glucosamine·HCl and echinocystic acid. Echinocystic acid: EI-MS: 472 [(*M*)⁺], 264 [(*M* – C₁₄H₂₄O)⁺], 246 [(264 – H₂O)⁺], 231 [(246 – CH₃)⁺], 219 [(264 – COOH)⁺], 208 [(C₁₄H₂₄O)⁺], 201 [(246 – COOH)⁺].

3-{[O- β -D-Galactopyranosyl-(1→4)-(2-acetamido-2-deoxy- β -D-glucopyranosyl)]oxy}olean-12-en-28-oic Acid (**3**). White amorphous powder. M.p. 255–258° (dec.). IR: 3400–3200 (OH), 2900, 1650, 1540 (CONH). $^1\text{H-NMR}$ (200 MHz): 0.79, 0.95, 0.98, 1.02, 1.18, 1.31 (21 H, 7 CH_3); 2.17 (NHCOCH₃); 3.8–5.10 (carbohydrate protons); 5.00 (unres., H–C(1'), H–C(1'')); 5.47 (br. *t*, H–C(12)); 8.98 (*d*, *J* = 9, NH). $^{13}\text{C-NMR}$ (50.1 MHz): Table 2. DCI-MS: 822 [(*M* + H)⁺], 660 [(*M* + H) – 162]⁺, 457 [(*M* + H) – 162 – 203]⁺, 204 [(203 + H)⁺].

Acidic hydrolysis of **3** afforded oleanolic acid, β -D-glucosamine·HCl, and galactose.

Enzymatic hydrolysis of **3** with galactosidase afforded a compound whose physical data were identical to **1**.

Peracetyl Derivative 3a. White amorphous powder. M.p. 225–230° (dec.). $^1\text{H-NMR}$ (200 MHz; see also Table 1): 0.72, 0.73, 0.89, 0.90, 1.10, 1.20, (21 H, 7 CH_3); 1.91, 1.97, 2.03, 2.08, 2.09, 2.14 (8 COCH₃); 3.5–5.50 (carbohydrate protons); 4.46 (*d*, *J* = 8, H–C(1')); 4.48 (*d*, *J* = 8, H–C(1'')); 5.23 (br. *t*, H–C(12)); 5.69 (*d*, *J* = 9, NH). DCI-MS: 1074 [(*M* + H)⁺], 1042 [(*M* + H) – 42]⁺.

3-{[O- β -D-glucopyranosyl-(1→6)-(2-acetamido-2-deoxy- β -D-glucopyranosyl)]oxy}olean-12-en-28-oic Acid (**4**). White amorphous powder. M.p. 228–230° (dec.). IR: 3400–3200 (OH), 2900, 1650, 1535 (CONH). $^1\text{H-NMR}$ (200 MHz): 0.79, 0.95, 0.98, 1.00, 1.12, 1.29 (21 H, 7 CH_3); 2.17 (NHCOCH₃); 3.8–5.10 (carbohydrate protons); 5.00 (*d*, *J* = 8, H–C(1')); 5.16 (*d*, *J* = 8, H–C(1'')); 5.42 (br. *t*, H–C(12)); 8.97 (*d*, *J* = 9, NH). $^{13}\text{C-NMR}$ (50.1 MHz): Table 2. DCI-MS: 822 [(*M* + H)⁺], 660 [(*M* + H) – 162]⁺, 457 [(*M* + H) – 162 – 203]⁺, 204 [(203 + H)⁺].

Acid hydrolysis of **4** afforded oleanolic acid, β -D-glucosamine·HCl, and glucose.

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