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

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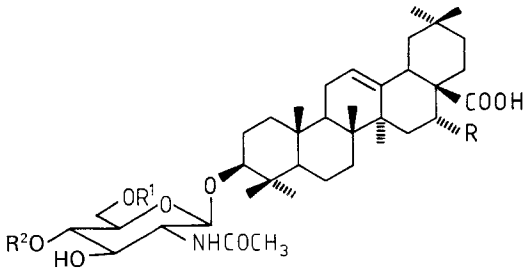
Activity-guided fractionation of the MeOH extract of the fruits of *Tetrapleura tetraptera* TAUB. (Mimosaceae) afforded 4 saponins **1**–**4**, which exhibited strong molluscicidal properties against the schistosomiasis-transmitting snails *Biomphalaria glabrata*. Chemical, enzymatic, and spectral methods (DCI-MS, $^1$H-NMR, $^{13}$C-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$\beta$-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 $CH_2Cl_2$, MeOH, and $H_2O$. The MeOH extract (80 g) which is molluscicidal at 25 ppm was then partitioned between $H_2O$ 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 4N 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 $H_2SO_4$ 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 ($NH_3$, 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 N*H* signal at *ca.* 9.0 ppm (*d*, *J* = 9, 1 H) in the $^1$H-NMR spectra of **1**–**4** was observed. The presence in all $^{13}$C-NMR spectra of a $CH_3$ 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 $^1$H-NMR data (H–C(1′) at 5.0 ppm (*J* = 8 Hz)).

The aglycone of **2** could be identified as echinocystic acid ( = 3β,16α-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]-16α-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

($[C_{14}H_{24}O]^{+\cdot}$), indicating the presence of an OH group at ring C, D, or E of the triterpene. Chemical shifts of the triterpenoid moiety in the $^{13}$C-NMR spectrum of **2** were in good agreement with a 16α-OH substitution pattern [9].

In addition, the $^{13}$C-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 $[M + H]^{+\cdot}$) identified as aridanin (**1**; $^1$H-NMR, $^{13}$C-NMR). Thus, galactose is the terminal sugar. The interglycosidic linkage was determined by comparison of the $^1$H-NMR spectra ($CDCl_3$) of the peracetylated derivatives **1a** and **3a** of saponins **1** and **3**, respectively, and by the $^{13}$C-NMR spectrum of **3**.

Thus, the structure of **3** is established as 3-{[*O*-β-D-galactopyranosyl-(1→4)-(2-acetamido-2-deoxy-β-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 ($[M + NH_4]^{+\cdot}$) and 822 ($[M + H]^{+\cdot}$). A peak at *m/z* 660 ($[(M + H) - 162]^{+\cdot}$), resulting from the elimination of a hexosyl moiety, could be observed.

Table 1. *$^1$H-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 | *d*, $J = 8$ | 4.46 | *d*, $J = 8.1$ |
| | H–C(2′) | 3.77 | *ddd*, $J = 8, 9, 9.5$ | 4.00 | $m \approx ddd$ |
| | H–C(3′) | 5.32 | *dd*, $J = 9.5, 9.5$ | 5.10 | *dd*, $J = 9, 9$ |
| | H–C(4′) | 5.00 | *dd*, $J = 9.5, 9.5$ | 3.72 | *dd*, $J = 9, 9$ |
| | H–C(5′) | 3.6–3.7 | *m* | 3.59 | *m* |
| | H–C(6′) | 4.06 | *dd*, $J = 12, 2$ | 4.0–4.20 | unres. |
| | H′–C(6′) | 4.24 | *dd*, $J = 12, 2$ | 4.4–4.50 | unres. |
| | NH–C(2′) | 6.00 | *d*, $J = 9$ | 5.60 | *d*, $J = 9$ |
| Peracetylated Gal | H–C(1″) | | | 4.50 | *d*, $J = 8$ |
| | H–C(2″) | | | 5.12 | *dd*, $J = 8, 10$ |
| | H–C(3″) | | | 4.96 | *dd*, $J = 10, 3$ |
| | H–C(4″) | | | 5.35 | *dd*, $J = 3, < 1$ |
| | H–C(5″) | | | 3.87 | *dd*, $J = 7, < 1$ |
| | H–C(6″), H′–C(6″) | | | 4.0–4.20 | unres. |

[a]) Spectra were measured in $CDCl_3$.

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

In the peracetates **1a** and **3a**, the $^1$H-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 $^1$H-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 $CH_2OAc$, $CH_2OR$, 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 $^{13}$C-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 $^1$H-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 ($[M + H]^{+\cdot}$), 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 $^{13}$C-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 (*t*) | 38.5 | 38.5 | 38.5 |
| C(2) | 26.2 (*t*) | 26.3 | 26.3 | 26.5 |
| C(3) | 89.2 (*d*) | 89.0 | 89.3 | 89.1 |
| C(4) | 39.2 (*s*) | 39.2 | 39.2 | 39.2 |
| C(5) | 55.7 (*d*) | 55.7 | 55.7 | 55.6 |
| C(6) | 18.5 (*t*) | 18.5 | 18.5 | 18.4 |
| C(7) | 33.2 (*t*) | 33.4 | 33.3 | 33.1 |
| C(8) | 39.7 (*s*) | 39.8 | 39.7 | 39.6 |
| C(9) | 48.0 (*d*) | 47.1 | 48.0 | 47.9 |
| C(10) | 36.9 (*s*) | 36.9 | 36.9 | 36.9 |
| C(11) | 23.6 (*t*) | 23.7 | 23.7 | 23.7 |
| C(12) | 122.5 (*d*) | 122.1 | 122.4 | 122.6 |
| C(13) | 144.8 (*s*) | 145.2 | 144.9 | 144.6 |
| C(14) | 42.0 (*s*) | 42.0 | 42.0 | 41.8 |
| C(15) | 28.3 (*t*) | 36.1 | 28.3 | 28.2 |
| C(16) | 23.7 (*t*) | 74.8 (*d*) | 23.7 | 23.7 |
| C(17) | 46.7 (*s*) | 49.0 | 46.7 | 46.5 |
| C(18) | 42.1 (*d*) | 41.5 | 42.1 | 42.0 |
| C(19) | 46.5 (*t*) | 47.3 | 46.5 | 46.6 |
| C(20) | 30.9 (*s*) | 31.0 | 30.9 | 30.9 |
| C(21) | 34.1 (*t*) | 36.1 | 34.2 | 34.1 |
| C(22) | 33.2 (*t*) | 32.5 | 33.2 | 33.1 |
| C(23) | 28.3 (*q*) | 28.0 | 28.0 | 28.0 |
| C(24) | 16.9 (*q*) | 16.9 | 16.9 | 16.9 |
| C(25) | 15.4 (*q*) | 15.5 | 15.3 | 15.3 |
| C(26) | 17.3 (*q*) | 17.5 | 17.4 | 17.3 |
| C(27) | 26.1 (*q*) | 27.2 | 26.1 | 26.1 |
| C(28) | 180.3 (*s*) | 180.0 | 180.5 | 180.2 |
| C(29) | 33.2 (*q*) | 33.4 | 33.2 | 33.3 |
| C(30) | 23.7 (*q*) | 24.9 | 23.7 | 23.7 |
| C(1′) | 104.5 (*d*) | 104.8 | 104.4 | 104.9 |
| C(2′) | 58.0 (*d*) | 57.8 | 57.4 | 57.7 |
| C(3′) | 76.1 (*d*) | 76.2 | 73.6 | 75.9 |
| C(4′) | 72.5 (*d*) | 72.5 | 83.5 | 72.5 |
| C(5′) | 78.1 (*d*) | 78.2 | 76.2 | 76.9 |
| C(6′) | 62.9 (*t*) | 62.8 | 62.5 | 70.3 |
| *C*OCH$_3$ | 170.2 (*s*) | 170.2 | 169.9 | 170.1 |
| CO*C*H$_3$ | 23.7 (*q*) | 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 (D$_5$)pyridine.

compared to **1**, see *Table 2*). Thus, **4** is 3-{[*O*-*β*-D-glucopyranosyl-(1→6)-(2-acetamido-2-deoxy-*β*-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 μm, *Merck,* Darmstadt). Reversed-phase chromatography: *Büchi B 681* MPLC system with a 2.6 × 46-cm column packed with *RP-8* material (15–25 μm, *Merck* No. *9324*). TLC: silica-gel-precoated Al sheets (*Merck*) with AcOEt/MeOH/$H_2O$ 17:2:1 *(syst. I)* and petroleum ether/AcOEt 1:1 *(syst. II)*, precoated *RP-8* nanoplates *(Merck)* with MeOH/$H_2O$ 7:3, detection with *Godin* reagent [20], or cellulose-precoated Al sheets (*Merck*) with AcOEt/pyridine/AcOH/$H_2O$ 5:5:1:3 *(syst. 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 × 45 cm *Sephadex-LH-20* column *(Pharmacia)* with MeOH or $CH_2Cl_2$/MeOH 1:2. M.p.: *Mettler FP 80/82* hot stage apparatus; uncorrected. IR spectra: *Philips PU 9700* spectrophotometer; in KBr. $^1$H-NMR and $^{13}$C-NMR spectra: *Varian VXR-200* equipped with switchable 5-mm probe at 200 and 50.1 MHz, resp., ($D_5$)pyridine solns. for saponins **1–4** (30 mg/0.8 ml), and $CDCl_3$ 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 ($NH_3$, positive-ion mode): *Nermag R1030* 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 $CH_2Cl_2$ (3 × 500 ml), MeOH (3 × 500 ml), and $H_2O$ (3 × 500 ml). The MeOH extract (80 g) was partitioned between $H_2O$ (500 ml) and BuOH (500 ml) and the BuOH extract (20 g) separated on a silica-gel column with $CHCl_3$/MeOH/$H_2O$ 85:11:1→70:30:5→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 $CH_2Cl_2$/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/$H_2O$ 7:3 afforded **2** (60 mg) and **3** (170 mg). Droplet counter-current chromatography (DCCC) of *Fraction 12* (1000 mg) with $CHCl_3$/MeOH/$H_2O$ 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 4N HCl (2 ml) for 2 h. The mixtures were then extracted with AcOEt. The org. layers were checked by TLC (silica gel, *syst. II*), HPLC (*RP-8,* $CH_3CN/H_2O$, 65:35), and EI-MS for aglycones. The aq. phases were neutralized with $NaHCO_3$ and the sugars extracted with pyridine and analyzed by TLC on silica gel with AcOEt/MeOH/$H_2O$/AcOH 65:15:15:20 (detection with *p*-anisidine phthalate) and on cellulose with *syst. III* (detection with ninhydrin). Saponin **2** (2 mg) was refluxed with 2N $H_2SO_4$ (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, *syst. I*). After 7 days, the mixture was extracted with 2 × 40 ml of BuOH and the org.

residue purified by silica-gel column chromatography using $CHCl_3$/MeOH 95:5: 15 mg of aridanin (**1**; TLC, HPLC, $^1$H-NMR, $^{13}$C-NMR).

*Acetylation.* Compounds **1** and **3** (15 mg each) were dissolved in $Ac_2O$/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 ($CH_2Cl_2$/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). $^1$H-NMR (200 MHz): 0.78, 0.97, 1.00, 1.02, 1.21, 1.31 (21 H, 7 $CH_3$); 2.17 (NHCOC$H_3$); 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). $^{13}$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.). $^1$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 $COCH_3$); 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 ($[M + NH_4]^+$), 786 ($[M + H]^+$).

*3-[(2-Acetamido-2-deoxy-β-*D*-glucopyranosyl)oxy]-16α-hydroxyolean-12-en-28-oic Acid* (**2**). White amorphous powder. M.p. 265–270° (dec.). IR: 3400–3200 (OH), 2900, 1650, 1535 (CONH). $^1$H-NMR (200 MHz): 0.82, 0.99, 1.02, 1.07, 1.19, 1.83 (21 H, 7 $CH_3$); 2.15 (NHCOC$H_3$); 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). $^{13}$C-NMR (50.1 MHz): *Table 2.* DCI-MS: 676 ($[M + H]^+$), 490 ($[472 + NH_4]^+$), 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 ($[M - C_{14}H_{24}O]^+$), 246 ($[264 - H_2O]^+$), 231 ($[246 - CH_3]^+$), 219 ([264–COOH]$^+$), 208 ($[C_{14}H_{24}O]^+$), 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). $^1$H-NMR (200 MHz): 0.79, 0.95, 0.98, 1.02, 1.18, 1.31 (21 H, 7 $CH_3$); 2.17 (NHCOC$H_3$); 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). $^{13}$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.). $^1$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 $COCH_3$); 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)]}oxyolean-12-en-28-oic Acid* (**4**). White amorphous powder. M.p. 228–230° (dec.). IR: 3400–3200 (OH), 2900, 1650, 1535 (CONH). $^1$H-NMR (200 MHz): 0.79, 0.95, 0.98, 1.00, 1.12, 1.29 (21 H, 7 $CH_3$); 2.17 (NHCOC$H_3$); 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). $^{13}$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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