60. Molluscicidal Saponins from *Talinum tenuissimum* DINTER[1])

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Dedicated to Prof. *K. Nakanishi* on the occasion of his 60th birthday

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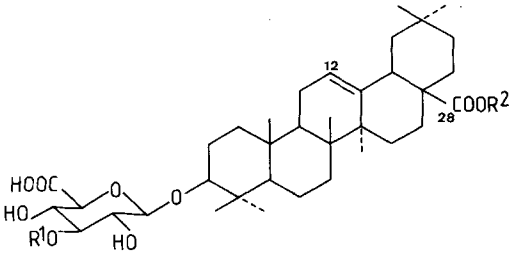
Two new saponins, β-D-glucopyranosyl 3-*O*[*O*-β-D-xylopyranosyl-(1→3)-O-(β-D-glucopyranosyluronic acid)]oleanolate (**1**) and 3-*O*-[*O*-β-D-xylopyranosyl-(1→3)-O-(β-D-glucopyranosyluronic acid)]oleanolic acid (**2**), have been isolated from the tubers of *Talinum tenuissimum*. The structures have been established mainly by ^{13}C-NMR and FAB-MS. The monodesmosidic saponin **2** exhibits very strong molluscicidal activity against the schistosomiasis-transmitting snail *Biomphalaria glabrata*.

Introduction. – The tubers of *Talinum tenuissimum* DINTER *(Portulacaceae)* are used in Malawi, according to traditional healers, for the treatment of schistosomiasis. In the course of our systematic screening studies on compounds with molluscicidal activity from medicinal plants [1–3], we noticed that the H_2O extract of *T. tenuissimum* also killed *Biomphalaria glabrata* snails, the intermediate host of *Schistosoma mansoni*, at a concentration as low as 25 ppm within 24 h. This observation prompted us to undertake the phytochemical investigation of this species which has not been studied before. Furthermore, it appears that very little is known about the constituents of the genus *Talinum* [4] [5]. In the present paper, we report on the isolation of new triterpenoid saponins which are responsible for the molluscicidal activity of the crude plant extract.

Results and Discussion. – Fresh tubers (400 g) collected near Zomba, Malawi, were extracted with MeOH and yielded 21 g of extract. A part of this extract (11.5 g) was suspended in H_2O and partitioned with $CHCl_3$ and BuOH. The active BuOH extract (3.6 g) was submitted to flash chromatography [6] on silica gel with $CHCl_3$/MeOH/H_2O 65:40:5, followed by preparative reversed-phase chromatography on *RP-8* with MeOH/H_2O mixtures and afforded finally 850 mg of compound **1** and 10 mg of compound **2**.

Acidic hydrolysis of **1** and **2** afforded the same aglycone, identified as oleanolic acid by comparison with an authentic sample (TLC, ^{13}C-NMR, MS). The sugar obtained



Compound	R[1a])	R[2b])
1	Xyl	Glc
2	Xyl	H
3	H	H

[a]) Xyl: β-D-xylopyranosyl.
[b]) Glc: β-D-glucopyranosyl.

[1]) Part 3 of the series 'Phytochemistry of African Medicinal Plants'. For part 2, see [1].

from the saponin hydrolysates were D-xylose, D-glucose, and D-glucuronic acid from **1** and D-xylose and D-glucuronic acid from **2**. Basic hydrolysis of **1** yielded D-glucose and the monodesmosidic saponin **2**. Mild acidic hydrolysis (0.1N HCl) of **2** afforded D-xylose and 3-*O*-(*β*-D-glucopyranosyluronic acid) oleanolic acid **3** identified by comparison with an authentic sample previously isolated from *Lonicera nigra* L. [7]. Thus, compound **2** is a monodesmosidic saponin of oleanolic acid with a xylosyl-(glucosyluronic acid) moiety and **1** is a bidesmosidic saponin with an additional glucosyl unit attached through an ester bond at C(28) of the aglycone.

The mol. wt. and the sugar sequence of the saponins were established by fast atom bombardment (FAB) MS [8]. The MS of **1** (thioglycerol matrix, negative-ion mode) showed a quasi-molecular ion at *m/z* 925 ($[M - H]^-$) and signals at *m/z* 793 ($[(M - H) - 132]^-$), 763 ($[(M - H) - 162]^-$), and 631 ($[(M - H) - 294]^-$) corresponding to the loss of a xylosyl moiety, a glucosyl moiety, and a loss of both sugar moieties, respectively. In the MS of **2**, quasi-molecular ions appeared at *m/z* 786 ($[(M + Na) - H]^-$) and 763 ($[M - H]^-$). Signals at *m/z* 631 ($[(M - H) - 132]^-$) and 455 ($[(M - H) - 308]^-$) corresponded to the subsequent loss of a xylosyl moiety and a glucuronic-acid moiety and indicated clearly that xylose was the terminal sugar.

The interglycosidic linkages as well as the position of attachment of the sugar chains to the aglycone in the saponins **1–3** were established by ^{13}C-NMR spectroscopy. The ^{13}C-NMR data which allowed the determination of the interglycosidic linkages are summarized in the *Table*.

In both saponins **1** and **2**, the xylosyl-(glucosyluronic acid) moiety was linked to the aglycone at C(3); C(3) of **1** and **2** appeared at 89.7 and 89.4 ppm, respectively, whereas in oleanolic acid, this C-atom was observed at 78.8 ppm. The free COOH-group appeared at 180.2 ppm (monodesmosidic saponin **2**), whereas when esterified with a glucosyl moiety, the chemical shift was 175.7 ppm (bidesmosidic saponin **1**). The *β*-D-configurations of glucose, xylose, and glucuronic acid in compound **1** were established by comparison of the sugar C(1) chemical shifts (see *Table*) with published data for *β*-D-glucopyranosyl 3*β*-*O*-[*O*-*β*-D-xylopyranosyl-(1→2)-*O*-(*β*-D-glucopyranosyluronic acid)]pfaffate [9] and for hovenoside [10].

Table. *^{13}C-NMR Chemical Shifts of Saponins* **1–3** *(Sugar Moieties Attached to the Aglycone at C(3))*

Saponin		C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
1	(glucosyluronic acid) moiety	105.4	73.4[a])	85.9	73.4[a])	78.2	172.3
	xylosyl moiety	106.0	73.6[a])	77.0	70.9	66.6	–
2	(glucosyluronic acid) moiety	106.2	74.6	86.6	71.5	78.0	172.2
	xylosyl moiety	106.8	75.2	77.4	71.0	67.4	–
3	[9] (glucosyluronic acid) moiety	107.1	75.4	78.0[b])	73.4	77.7[b])	173.3

[a])[b]) Attributions may be interchanged.

The chemical shifts of the C-atoms of the (*β*-D-glucopyranosyluronic acid) moiety of **1** and **2** clearly indicated that the terminal *β*-D-xylopyranosyl unit is attached at position 3 *(see Table)*. Compared with **3**, the C(3)-signal was shifted downfield by 7.9 ppm to 85.9 ppm in **1** and by 8.6 ppm to 86.6 ppm in **2**. Small upfield shifts were observed for the C(2)- and C(4)-signals, whereas the other C-atoms remained almost unaffected. These results are in agreement with the glycosylation rule previously established by *Konishi et al* [11]. Furthermore, the chemical shifts of the (*β*-D-glucopyranosyluronic acid) moiety of **1** and **2** correspond to those of 3-*O*-[*O*-*α*-D-arabinopyranosyl-(1→3)-*O*-(*β*-D-glucopyranosyluronic acid)]oleanolic acid [12].

Thus, the structure of **1** is established as *β*-D-glucopyranosyl 3-*O*-[*O*-*β*-D-xylopyranosyl-(1→3)-*O*-(*β*-D-glucopyranosyluronic acid)]oleanolate and that of **2** as 3-*O*-[*O*-*β*-D-

xylopyranosyl-(1→3)-*O*-(β-D-glucopyranosyluronic acid)]oleanolic acid. Both are new natural products. However, it is noteworthy that an isomer of **2** with a 1→2 interglycosidic linkage has recently been described [12]. TLC comparison of **2** with an authentic sample of its isomer, kindly supplied by Prof. *O. Tanaka,* showed that both compounds were different.

The major bidesmosidic saponin **1** isolated from the MeOH extract was inactive against *Biomphalaria glabrata* snails, whereas the monodesmosidic saponin **2** killed the snails at a concentration of 1.5 ppm within 24 h. The MeOH extract was active at 400 ppm but was fully inactive at further dilutions. Direct H_2O extraction afforded a highly active solution killing the snails at a concentration of 25 ppm. The H_2O extract contained mainly saponin **2** and only traces of **1** could be detected. Thus, the molluscicidal activity of saponin-containing plants depends on the extraction process since the genuine inactive bidesmosidic saponins are easily hydrolyzed to very active monodesmosidic saponins in the course of the H_2O extraction. A similar observation was made during the study of *Phytolacca dodecandra* L'HÉRIT *(Phytolaccaceae)* [3]. *T. tenuissimum* contains further saponins, devoid of molluscicidal activity. Their structure elucidation is currently in progress.

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Experimental Part

General Remarks. Melting points (m.p.) were determined on a *Kofler* block and are uncorrected. TLC were carried out on silica-gel-precoated Al sheets *(Merck)* with $CHCl_3$/MeOH/H_2O 65:40:10 and on *RP-8*-precoated glass plates (HP TLC, *Merck*) with MeOH/H_2O 60:40. Detection was with *Godin* reagent [13]. R_f values (silica gel): 0.15(**1**), 0.25(**2**), and 0.29(**3**). For flash chromatography [6], a 4 × 60–cm column with 280 g of silica gel *60* (63–200 μm, *Merck*) was used. Reversed-phase separations were achieved on a *Jobin-Yvon-Chromatospac* apparatus, equipped with a 4 × 50–cm column (200 g of *RP-8 Lichroprep,* 15–25 μm; *Merck*) and *Lobar-Lichroprep-RP-8* columns (40–63 μm; 2.5 × 27 cm; *Merck*) equipped with a *Duramat-80* pump (*Chemie und Filter,* Regensdorf). *Sephadex-LH-20 (Pharmacia Fine Chemicals)* separations were carried out on a 2.5 × 50–cm column. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a *Bruker WP-360* (360 and 90.52 MHz) and on a *Bruker WP-200* (200 and 50.29 MHz) apparatus in (D_5)pyridine as solvent and TMS as internal standard. Fast atom bombardment (FAB) MS were obtained on a *ZAB-1S* spectrometer. The target was bombarded with 5-keV Xe-atoms; samples were suspended in thioglycerol. Specific-rotation values were measured on a *Zeiss* polarimeter in 5-ml tubes. Bioassays were made with snails of the species *Biomphalaria glabrata* as described previously [14].

Acidic Hydrolysis. The saponin (4 mg) in MeOH (1 ml) was refluxed in 1N HCl (20 ml) for 4 h. The aglycone was extracted with Et_2O and identified by TLC on silica gel with (i-Pr)$_2$O/acetone 75:30. The aq. layer was adjusted to pH 3 with $NaHCO_3$. After evaporation to dryness, the sugars were extracted with pyridine from the residue and analyzed by TLC on silica gel with AcOEt/MeOH/H_2O/AcOH 95:15:15:20, detection with *p*-anisidine phthalate.

Basic Hydrolysis. As in [3]. The saponin (160 mg), from basic hydrolysis, in MeOH (8 ml) was deionised with ion-exchange resin *(Amberlite 200).*

Partial Hydrolysis. The saponin (120 mg) in MeOH (10 ml) was refluxed in 0.1N HCl (100 ml) for 12 h, and the soln. was extracted with H_2O-sat. BuOH (2 × 100 ml); the org. phase was washed with H_2O and evaporated to dryness. The crude mixture was separated on a small silica-gel column (20 g of silica gel *60,* 40–63 μm; *Merck*) with $CHCl_3$/MeOH/H_2O 70:30:5 to obtain the partially hydrolysed saponin in pure state.

Isolation. The roots of *Talinum tenuissimum* DINTER *(Portulacaceae)* were collected near Zomba, Malawi. Fresh roots (400 g) were extracted with MeOH to give 21 g of extract. A part of the crude extract (11.5 g) was suspended in H_2O (200 ml) and partitioned with $CHCl_3$ (2 × 100 ml) and BuOH (3 × 200 ml). The BuOH layer was evaporated to dryness (5.5 g). A part of the molluscicidal BuOH extract (3.6 g) was separated by flash chromatography with $CHCl_3$/MeOH/H_2O 65:40:5→65:50:10. The eluate was analysed by TLC and collected in 4 fractions. Only *Fraction II* (144 mg) showed molluscicidal activity (25 ppm/24 h). This bioactive fraction was submitted to reversed-phase chromatography *(Lobar RP-8)* with MeOH/H_2O 55:45 (3 ml/min) yielding 10 mg of pure **2**. *Fraction IV* (2.7 g) was submitted to prep. reversed-phase chromatography with MeOH/H_2O 6:4 (6 ml/min) giving 850 mg of **1**, which was purified on *Sephadex LH 20* (MeOH). The purity was checked by anal. HPLC on a *RP-8* column *(Knauer)* using H_2O/MeCN 7:3 (1.5 ml/min; detection at 206 nm).

Basic hydrolysis of **1** (200 mg) afforded 160 mg of prosapogenin, which was identical to saponin **2**. The prosapogenin (120 mg) was submitted to a partial acidic hydrolysis in 0.1N HCl. The mixture of completely hydrolysed, partially hydrolysed, and unhydrolysed saponins was separated on silica gel with $CHCl_3$/MeOH/H_2O 70:30:5 to afford pure saponin **3** (32 mg).

*β-D-Glucopyranosyl 3-O-[O-β-D-Xylopyranosyl-(1→3)-O-(β-D-glucopyranosyluronic acid)]oleanolate (= β-D-Glucopyranosyl 3β-{[O-β-D-Xylopyranosyl-(1→3)-O-(β-D-glucopyranosyluronic acid)]oxy}olean-12-en-28-oate; **1**):* White powder, m.p. 270–75° (dec.), $[\alpha]_D^{22} = 4°$ (MeOH, $c = 1$). ^{13}C-NMR (90.52 MHz, (D_5)pyridine): δ's of the aglycone correspond to those of oleanolic acid [15]; sugar signals: xylose and glucuronic acid, see *Table;* glucose: 95.1 (C(1)); 73.6 (C(2)); 78.4 (C(3)); 70,7 (C(4)); 78.2 (C(5)); 61.7 (C(6)). MS (FAB, negative ions, thioglycerol): 925 ($[M - H]^-$), 763 ($[(M - H) - 162]^-$), 793 ($[(M - H) - 132]^-$), 631 ($[(M - H) - 294]^-$).

Acidic hydrolysis of **1** afforded oleanolic acid, D-glucose, D-xylose and D-glucuronic acid. Basic hydrolysis afforded D-glucose and the prosapogenin **2**.

*3-O-[O-β-D-Xylopyranosyl-(1→3)-O-(β-D-glucopyranosyluronic acid)]oleanolic Acid (= 3β-{[O-β-D-Xylopyranosyl-(1→3)-O-(β-D-glucopyranosyluronic acid)]oxy}olean-12-en-28-oic Acid; **2**):* White powder, m.p. 235–40° (dec.), $[\alpha]_D^{22} = 12°$ (MeOH, $c = 0.5$). ^{13}C-NMR (50.29 MHz, (D_5)pyridine): δ's of the aglycone correspond to those of oleanolic acid [15]; sugar signals, see *Table.* MS (FAB, negative ions, thioglycerol): 786 ($[M + Na) - H]^-$), 763 ($[M - H]^-$). 631 ($[(M - H) - 132]^-$), 455 ($[(M - H) - 308]^-$).

Acidic hydrolysis of **2** afforded oleanolic acid, D-xylose and D-glucuronic acid.

*3-O-(β-D-Glucopyranosyluronic acid)oleanolic Acid(= 3β-[(β-D-Glucopyranosyluronic acid)oxy]olean-12-en-28-oic Acid; **3**):* White powder, m.p. 242–244° (dec.). MS (FAB, negative ions, thioglycerol): 631 ($[M - H]^-$), 455 ($[(M - H) - 176]^-$). Saponin **3** was previously described in [7].

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