Molluscicidal Triterpenoid Glycosides of *Dialium guineense*

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The molluscicidal activity of the fruit and leaves of *Dialium guineense* was found to be due to oleanolic acid saponins, three of which were isolated from the fruit and a fourth from the leaves. The presence of these known saponins in *Dialium* spp. have not been previously reported, and the molluscicidal activity of 3-*O*-[*β*-D-xylopyranosyl]oleanolic acid is here reported for the first time.

Dialium guineense Willd. (Leguminosae) is known as "Awin" in the southwest Benin district of Nigeria, and the fruits and leaves are sold in markets as the ingredients for a refreshing drink when mixed with water. The plant is used in the coastal regions of West Africa for a variety of medicinal purposes including dysmenorrhoea, fever, labor pains, and palpitations.[1] In spite of these interesting properties no phytochemical investigation of any member of the genus *Dialium* has been reported.

Screening of a random collection of Nigerian local plants for molluscicidal activity showed that aqueous and methanolic extracts of the leaves and fruits of *D. guineense* gave 100% mortality at a concentration of 100 ppm within 24 h when tested against *Biomphalaria glabrata*.[2,3] This snail is the vector involved in the transmission of *Schistosoma mansoni*, which is a causative organism of bilharzia. In recent years research has been carried out to discover local plants with molluscicidal activity in areas where bilharzia is common with a view to economic disease prevention without recourse to imported materials.

Extracts of other members of the Leguminosae, e.g., *Swartzia madagascariensis* Desv., have also shown this activity, which has been ascribed to the triterpenoid saponins present.[4−6] These have been shown to be as effective as synthetic molluscicides and have been used in the field with encouraging results.[7,8] *D. guineense* complies with the criteria specified for an effective natural molluscicide[9] since it is a common plant, the activity is located in the regenerating parts, it is culturally acceptable, and a good knowledge of its growing habits exists. It was therefore considered worthwhile to investigate the identity and activity of the compounds responsible for the observed molluscicidal activity since this would be necessary before the plant could be used in public health measures.

The saponins isolated were identified as the known compounds 3-*O*-[*β*-D-glucopyranosiduronic acid]oleanolic acid,[10−13] 3-*O*-[*β*-D-xylopyranosyl(1→3)-*β*-D-glucopyranosiduronic acid]oleanolic acid,[14] 3-*O*-[α-L-rhamnopyranosyl(1→3)-*β*-D-glucopyranosiduronic acid]oleanolic acid,[4,5,15−17] and 3-*O*-[*β*-D-xylopyranosyl]oleanolic acid.[18] The parent aglycon oleanolic acid was also isolated. Identification was by comparison with literature values for the mass and NMR spectroscopic data for the saponins and oleanolic acid and, after hydrolysis, by GLC and TLC chromatographic comparison of the sugars and oleanolic acid with authentic substances. This is the first report of any compounds to be isolated from *Dialium guineense*.

The molluscicidal activity (Table 1) was confined to the glycosidal compounds since the parent aglycon oleanolic acid displayed no activity. There appears to be some correlation between activity and the presence of a glucuronic acid in the sugar part of the molecules since 3-*O*-[*β*-D-xylopyranosyl]oleanolic acid, which did not contain this residue, had a lower activity than the other saponins. This observation is in accord with structure−activity observed for similar triterpenoid glycosides.[19] The activities reported here compare well with previous studies carried out on the three glucuronic acid-containing saponins 3-*O*-[*β*-D-glucopyranosiduronic acid]oleanolic acid, 3-*O*-[*β*-D-xylopyranosyl(1→3)-*β*-D-glucopyranosiduronic acid]oleanolic acid, and 3-*O*-[α-L-rhamnopyranosyl(1→3)-*β*-D-glucopyranosiduronic acid]-oleanolic acid, which gave activities of 2.0, 3, and 3 ppm, respectively.[4,5,15] The molluscicidal activity of 3-*O*-[*β*-D-xylopyranosyl]oleanolic acid is being reported for the first time.

D. guineense has been shown to contain up to 15% w/w total saponins in the leaves and fruits[20] and thus could provide a useful local source of a molluscicidal crude extract to control *B. glabrata*. Its potential is augmented by the fact that the leaves are available throughout the year and also that the tree produces prolific amounts of fruit which can be dried and stored without significant loss of saponin content.[20]

Experimental Section

General Experimental Procedures. Melting points were determined using an electrothermal melting point

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Table 1. Molluscicidal Activity of Isolated Compounds

compd	molluscicidal activity[a] (ppm)
3-*O*-[β-D-glucopyranosiduronic acid]oleanolic acid	2.0 ± 0.4
3-*O*-[β-D-xylopyranosyl(1→3)-β-D-glucopyranosiduronic acid]oleanolic acid	4.0 ± 0.6
3-*O*-[α-L-rhamnopyranosyl(1→3)-β-D-glucopyranosiduronic acid]oleanolic acid	4.0 ± 0.3
3-*O*-[β-D-xylopyranosyl]oleanolic acid	>16.0
oleanolic acid	0.0

[a] Minimum concentration causing 100% mortality as shown by lack of heart bet and no recovery after 24 h.

apparatus and are uncorrected. The EIMS were recorded on an AEI 902 high-resolution mass spectrometer at 130 °C using a direct inlet; FABMS were determined in a negative ion mode in a thioglycerol matrix bombardment with xenon gas. ^{1}H NMR and ^{13}C NMR spectra were recorded on a VLIRS AMX 400 MHz spectrometer. TMS was used as the internal standard for all spectra. TLC was performed using silica gel $60F_{254}$. Visualization of compounds was achieved for the triterpenoids by using CH_3COOH:H_2SO4:MeOH 10:5:85 followed by heating at 105 °C for 10 min and for the sugars by using 0.5% w/v *p*-anisidine reagent. GLC was carried out using a Shimadzu GC-8A chromatograph on 10 m × 0.5 mm NONPAXD column coated with AT-210 2.1 μm thick, flow rate temperature 150 °C rising 6°/min to 200 °C. Detector FID.

Collection and Extraction of Plant Material. The leaves and fruits of *D. guineense* were collected at Ile−Ife, Nigeria in May 1989 along Oba Aderemi Road, Obafemi Awolowo University, and identified by Prof. Z. Gbile by comparison with herbarium specimens FHI 6756, FHI 24636, FHI 32225, and FHI 2819 of the herbarium of the Forestry research Institute of Nigeria, Ibadan. Voucher specimens PHB 204, PHB 205, and PHB 206 are deposited in the herbarium of the Pharmacognosy Department, University of Benin, Nigeria.

Isolation and Identification of Compounds. Dried powdered fruit of *D. guineense* (50 g) was extracted with 1 L of cold H_2O for 24 h and filtered. The filtrate was freeze-dried to give 12.6 g of extract. The extract (10.6 g) was suspended in H_2O (300 mL) and the suspension partitioned with *n*-BuOH (3 × 200 mL). The combined BuOH extracts were evaporated to dryness to give a saponin fraction (6.4 g). This fraction (2.7 g) was subjected to flash LC (silica gel ICN Si 18-32, 60 Å) using a mobile phase gradient $CHCl_3$:MeOH:H_2O 65:35:5 to MeOH alone. Fractions were monitored by TLC and like fractions bulked, evaporated to dryness, and subjected to analysis for molluscicidal activity. The three major active fractions A (270 mg), B (182 mg), and C (216 mg) were each purified by reversed-phase VLC (LiChroprep RP-18/MeOH:H_2O 7:4) to yield 3-*O*-[β-D-glucopyranosiduronic acid]oleanolic acid (68 mg), 3-*O*-[β-D-xylopyranosyl(1→3)-β-D-glucopyranosiduronic acid]-oleanolic acid (35 mg), and 3-*O*-[α-L-rhamnopyranosyl(1→3)-β-D-glucopyranosiduronic acid]oleanolic acid (41 mg).

Dried powdered leaves of *D. guineense* (50 g) were treated in a similar way to the fruit to yield 10.8 g of dry crude extract and 4.3 g of crude saponin. The crude saponin (2.4 g) was fractionated on silica to give one major bioactive fraction which after RP purification afforded 3-*O*-[β-D-xylopyranosyl]oleanolic acid (26 mg).

Hydrolysis and Sugar Analysis. Saponins were hydrolyzed by acid, alkaline, and enzyme using 4 M HCl, 1 M KOH, and β-glucuronidase in the usual manner; details are available from the authors. Sugar fractions (5 mg each) from the acid hydrolysis and the reference sugars were reacted with 1 mL of Trisil reagent,[21] shaken vigorously, and allowed to stand at 25 °C for 15 min, and the resulting mixture was analyzed by GLC.

Test for Molluscicidal Activity. An adaptation of the WHO method[22] was performed. In brief, *B. glabrata* snails were reared in aquaria with a continuous circulation of dechlorinated water maintained at 28 °C through a filter system. Snails of uniform size (diameter of shell ~7.5 mm) were used. For each test five snails were placed in 100 mL of dechlorinated water with a known concentration of the extract or compound under test. In cases where the compound was not soluble directly in water, it was dissolved in a small amount of EtOH that was then made up to volume with dechlorinated water to produce a 1% EtOH solution.

Control groups of snails were placed in dechlorinated water or in a 1% EtOH solution. The snails were kept at 20 °C for 24 h and then taken out from the small tanks and placed on a Petri dish. Light was shone from the bottom of the dish, and each snail examined for the presence of heart beat. Snails showing no heart beat were placed for 24 h in dechlorinated water and then reexamined to check mortality or recovery, the latter being demonstrated by observance of heart beat.

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