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FUNGICIDAL AND MOLLUSCICIDAL SAPONINS FROM *DOLICHOS KILIMANDSCHARICUS*

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Key Word Index—*Dolichos kilimandscharicus*, Leguminosae, saponins, fungicides; molluscicides

Abstract—Three saponins with molluscicidal and fungicidal activities have been isolated from the roots of *Dolichos kilimandscharicus*. They were shown to be the 3-*O*-β-D-glucopyranosides of hederagenin, bayogenin and medicagenic acid

INTRODUCTION

As part of an investigation into the molluscicidal and fungicidal properties of African medicinal plants, it was observed that a methanol extract of *Dolichos kilimandscharicus* Taub. (Leguminosae) roots was active in both bioassays. Infusions of the roots are taken in African traditional medicine for the treatment of dysentery [1] but no phytochemical investigation of *D. kilimandscharicus* has yet been undertaken. Apart from the study of lectins, amino acids and proteins from certain species of *Dolichos*, very little is known about the chemical constituents of the genus as a whole.

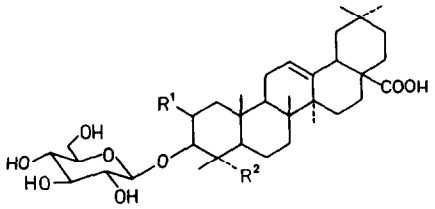
RESULTS AND DISCUSSION

Roots of *D. kilimandscharicus* were first extracted with dichloromethane and then with methanol. TLC analysis of the methanol extract showed the presence of saponins and the extract was separated by flash column chromatography into several fractions. One of the earlier fractions was then chromatographed by droplet countercurrent chromatography (DCCC). Subsequent low-pressure liquid chromatography (LPLC) and gel filtration (Sephadex LH-20) gave pure saponins **1** and **2**. A later fraction from flash chromatography yielded saponin **3** after LPLC on a reversed-phase (RP-8) support.

Acid hydrolysis of **1**–**3** gave glucose alone, identified by TLC, in all three cases. Compound **1** gave an aglycone corresponding in all respects to hederagenin (TLC, NMR, mp). The D/CI-mass spectrum of **1** gave a quasimolecular ion at *m/z* 652 $[M+NH_4]^+$, confirming **1** to be hederagenin 3-*O*-β-D-glucopyranoside.

The aglycone of **2** was shown to be bayogenin by TLC, NMR and mp comparisons with an authentic sample [2]. The quasimolecular ion in the D/CI mass spectrum appeared at *m/z* 668 $[M+NH_4]^+$. Thus, **2** was bayogenin 3-*O*-β-D-glucopyranoside.

Fast atom bombardment mass spectrometry (FABMS) of **3** in the positive ion mode gave a quasimolecular ion at *m/z* 687 $[M+H]^+$. The identity of the aglycone as medicagenic acid (2β,3β-dihydroxyolean-12-en-23,28-dioic acid) was confirmed by TLC and ^{13}C NMR spectroscopy. Saponin **3** was established as medicagenic acid 3-*O*-β-D-glucopyranoside. Substitution by glucose was shown in all three saponins to be at position 3 by ^{13}C NMR.



1 R^1 = H, R^2 = CH_2OH
2 R^1 = OH, R^2 = CH_2OH
3 R^1 = OH, R^2 = COOH

Table 1. Molluscicidal and fungicidal activities of saponins isolated from *Dolichos kilimandscharicus*

Saponin	Molluscicidal activity (mg/l)*	Antifungal activity (μg)†
1	15.0	5.0
2	7.5	2.5
3	25.0	5.0

**Biomphalaria glabrata* snails [3].

†Minimum amount required to inhibit *Cladosporium cucumerinum* spore formation in a TLC bioassay [4].

The three triterpene glycosides isolated from *D. kilimandscharicus* possessed noticeable activity both against the mollusc *Biomphalaria glabrata* [3](important as intermediate host in the tropical disease schistosomiasis) and against the fungus *Cladosporium cucumerinum* [4](see Table 1).

Only rarely have saponins been isolated from *Dolichos* species but there have been some reports on the saponins

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from *D. falcatus*. Medicagenic acid 3-*O*-β-D-glucopyranoside has recently been obtained from the roots of this plant in the form of a potassium salt [5] Hederagenin 3-*O*-β-D-glucopyranoside is known to occur, for example, in *Hedera helix* berries [3] and *H nepalensis* stem and bark (Araliaceae) [6]. It is unusual in saponin-containing plants to have different aglycones with the same sugar moiety; more common is the existence of saponins with one aglycone and variations in the saccharide chain. Although not toxic to freshwater snails, medicagenic acid from alfalfa (*Medicago sativa*) roots is known to have fungistatic effects against a number of fungal strains, including *Trichoderma viride* and *Aspergillus niger* [7] While this is the first report of molluscicidal activity of *Dolichos* saponins, the saponins from *D falcatus* have been reported to possess analgesic [8] and antitumour activities [9]

EXPERIMENTAL

General TLC was carried out on precoated silica gel A1 sheets (Merck) with $CHCl_3$-MeOH-H_2O (50.16 1) and on RP-8 precoated glass plates (HPTLC, Merck) with MeOH–H_2O (7 3) Detection was with Godin reagent [10] Flash chromatography [11] was performed on silica gel 60 (63–200 μm; Merck), gel filtration on Sephadex LH-20 (Pharmacia) and reversed-phase chromatography on Lobar LiChroprep RP-8 columns (40–63 μm, 2 5 × 27 cm, Merck), equipped with Duramat 80 pumps (Chemie and Filter), flow rate 3ml/min DCCC (DCC Chromatograph 670, Buchi, 283 columns, 2 7 × 400 mm, 50 ml/hr) was with the solvent system $CHCl_3$–MeOH–H_2O (7 13 8) in the descending mode ^{13}C NMR spectra were recorded in C_5D_5N using TMS as an int standard Bioassays were carried out with *Biomphalaria glabrata* snails [3] and *Cladosporium cucumerinum* spores [4] Acidic and basic hydrolyses were as previously described [2]

Plant material Roots of *D kilimandscharicus* were collected in June 1986 in Kenya A voucher specimen is kept at the Nairobi University Herbarium

Extraction and isolation Dried and powdered roots of *D kilimandscharicus* (50 g) were extracted with CH_2Cl_2 (1 l), then MeOH (2 × 1 l) Fraction II (420 mg) from flash chromatography ($CHCl_3$–MeOH–H_2O 50.10 1) of the MeOH extract (3.3 g) was subjected to DCCC separation The eluate was divided into fractions IIa, IIb and IIc Fraction IIa (80 mg) was further purified on a Lobar RP-8 column with MeOH–H_2O 75 25, yielding **1** (16 mg) Fraction IIb (100 mg) was filtered through a Sephadex LH-20 column (MeOH), to give **2** (18 mg). Fraction III (1 2 g) after the flash separation, was purified by gel filtration (MeOH) The resulting fraction IIIb (130 mg) was chromatographed on a Lobar RP-8 column with MeOH–H_2O (4 1) affording **3** (60 mg)

Hederagenin 3-O-β-D-glucopyranoside (**1**) [6]. D/CIMS *m/z* 652 $[M+NH_4]^+$, 635 $[M+H]^+$ ^{13}C NMR values identical to those published [6]

Bayogenin 3-O-β-D-*glucopyranoside* (**2**) D/CIMS m/z 668 $[M+NH_4]^+$, 651 $[M+H]^+$ ^{13}C NMR δ 46 4 (C-1), 70 4 (C-2),), 82 8 (C-3), 42 0 (C-4), 48 5 (C-5), 18 0 (C-6), 33 2 (C-7), 39 8 (C-8), 47 6 (C-9), 36 9 (C-10), 23 8 (C-11), 122.4 (C-12), 144 9 (C-13), 42 3 (C-14), 28 3 (C-15), 23.8 (C-16), 46 6 (C-17), 42 7 (C-18), 44 0 (C-19), 30 9 (C-20), 34.2 (C-21), 32 9 (C-22), 65 2 (C-23), 15 0 (C-24), 17 2 (C 25), 17 6 (C-26), 26 2 (C-27), 180 5 (C-28), 34 2 (C-29), 23 9 (C-30), δ of sugar moiety 105 6 (C-1), 75 3 (C-2), 78 2 (C-3), 71 4 (C-4), 78 4 (C-5), 62 4 (C-6)

Medicagenin 3-O-β-D-*glucopyranoside* (**3**) [7] FABMS *m/z* 687 $[M+Na]^+$, 665 $[M+H]^+$ ^{13}C NMR δ 46 8 (C-1), 71 5 (C-2), 86 7 (C-3), 44 4 (C-4), 52 4 (C-5), 21 3 (C-6), 33 5 (C-7), 40 3 (C-8), 48 8 (C-9), 36 9 (C-10), 24 0 (C-11), 122 4 (C-12), 145 2 (C-13), 42 2 (C-14), 34 5 (C-15), 33 3 (C-16), 53 1 (C-17), 42 5 (C-18), 48 8 (C-19), 26 5 (C-20), 33 5 (C-21), 28 4 (C-22), 180 3 (C-23), 14 8 (C-24), 17.6 (C-25), 17 0 (C-26), 24 0 (C-27), 180 6 (C-28), 31 1 (C-29), 24 0 (C-30), δ of sugar moiety 106 0 (C-1), 75 4 (C-2), 78 2 (C-3), 70 5 (C-4), 77 9 (C-5), 62 7 (C-6)

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