Antifungal Triterpenoid Saponins from *Lecaniodiscus cupanioides*

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Abstract: Phytochemical investigation of the stems of *Lecaniodiscus cupanioides* Planch ( Sapindaceae) afforded two triterpenoid saponins identified as 3-O- [α-L-arabinofuranosyl-
(1→3)- α-L-rhamnopyranosyl- (1→2) - α- L-arabinopyranosyl-]- hederagenin (1) and 3-O-
[α- L-arabinopyranosyl- (1→3)- α-L-rhamnopyranosyl (1→2)- α- L-arabinopyranosyl-]-
hederagenin (2). The structures of the compounds were determined based on chemical
investigations and comprehensive NMR spectroscopic studies including 1H, 13C, DEPT,
COSY, HMQC, HMBC, MS and comparison with literature data. The compounds exhibited
antifungal activity against *C. albicans, C. neoformans* and *A. fumigatus.*

Keywords: *Lecaniodiscus cupanioides*, Sapindaceae, triterpenoid saponin, hederagenin,
antifungal activity

INTRODUCTION

Almost every culture within the wide diversity of the world’s population and habitats uses local
plants within its environs as medicines in one form or another. Chemical and biological investigations
of folkloric medicinal plants with the reputation of curative potential have provided the world with
many of the common clinical drugs and herbal remedies of today (Hamburgher and Hostettmann, 1991).
It has been reported that several compounds derived from plant species could be regarded as important
drugs currently in use and that about 25% of the pharmaceuticals prescribed by doctors in the
developed world have their antecedents in chemicals produced by flowering plants (Balandrin et al.,
1985). There is also a growing interest in the acquisition of botanical enterprises by multinational
pharmaceutical companies in recent years (Leaders, 1996).

*Lecaniodiscus cupanioides* Planch ( Sapindaceae) is a tree sometimes planted as a shade-tree
and as an ornamental. It appears as a weed in rice fields in Nigeria. The bark is used for cough and
broncho-pneumonial infections (Burkill, 2000). Ethnobotanical information revealed its use as
galaetogenic, laxative and febrifuge and has autonomic effects such as larcination and skeletal muscle
relaxant activity in rats (Sandberg and Croulind, 1977). It is also used as an aphrodisiac and cases of
sexual asthma (Ghana Herbal Pharmacopoeia, 1992). The aqueous root extract of this plant was
reported to have central nervous system depressant activity (Yemitan and Adeyemi, 2005).
Preliminary screening of extracts from marine organisms and plants for antifungal activities in our
laboratory revealed that the stem extract of *L. cupanioides* had promising antifungal properties.
Incidence of fungal infection is increasing worldwide and despite treatment, mortality remains very
high. Presently, few antifungal agents are available and their use may be limited by harmful side effects
(Lorthoraly et al., 1999; Andriele, 1999). The present study thus deals with the isolation and
characterization of the saponins from *L. cupanioides* and their antifungal effects.

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MATERIALS AND METHODS

$^1$H and $^{13}$C-NMR Spectra were measured and reported in ppm by using the residual solvent peak as an internal standard. ESI-FTMS analysis was measured on a Bruker-Magex BioAPEX 30ES ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interphase. Semi-preparative HPLC was carried out on a Waters 510 system with a gradient programmer.

Plant Material

The stems of *L. cupanioides* were collected at Sango, Ogun State, Nigeria and authenticated by Mr. Odebo of the Forestry Research Institute of Nigeria (FRIN) Ibadan, voucher specimen FHI 105, 353 was deposited at the herbarium.

Extraction and Isolation

The stems of *L. cupanioides* were chopped into small bits and dried using an electric oven at 40°C for 3 days. The dried stems were ground in a roller mill. About 1 kg of powdered plant material was extracted exhaustively using Soxhlet apparatus with 96% ethanol (4 L) for 48 h. The filtrate was concentrated under reduced pressure using rotary evaporator until a semi-solid residue (5.1% w/w) was obtained. The extract was subjected to vacuum liquid chromatography over Si gel column, eluted successively with n-hexane-CHCl$_3$-MeOH in a step gradient by using different ratios to give eleven fractions (V1-V11). Fraction V8 (4.3 g) obtained on elution with CHCl$_3$-MeOH (7:3) was chromatographed over a Si gel column (4.0×70 cm) (EIOAc-Acetone-AcOH-H$_2$O 6:2:1:1) isocratically to give eleven subfractions (S1-S11). Fractions S8 (0.31 g) and S9 (0.06 g) gave semi-pure 1 and fractions S10 (0.07 g) and S11 (0.09 g) gave semi-pure 2 which were purified by preparative TLC (Merck 60Å Si gel, 20×20 cm, 1000 μm), developed with EIOAc-Acetone-AcOH-H$_2$O (6:2:1:1). Final purification was by reverse phase HPLC (Luna 5 μM, C8 100Å, 25×21.20 mm) using CH$_3$CN-H$_2$O (40:60) as an eluent (flow rate of 5 mL min$^{-1}$ gradiently and UV detection at 225 nm) yielded 15 mg of 1 (t$_R$ = 40 min) and 12 mg of 2 (t$_R$ = 40 min).

Compound 1

Colourless crystals; [α]$_D$$^{20}$+$^{11.2}$° (c 0.5, EtOH); ESI-HRMS m/z 882.3 ([M-H]$^-$); 750 [M-arabinose-H]$^-$; 604 [M-(arabinose+rhamnose)-H]$^-$; 471.3[M-(2arabinose+ rhamnose) -H]$^-$ (calc. for [C$_{60}$H$_{57}$O$_{10}$]$_n$ -1, 882.49767400).

$^1$H NMR (400.13 MHZ, C$_6$D$_5$N) δ 0.79 (3H, s, H-24), 0.80 (3H, s, H-26), 0.86 (3H, s, H-29), 0.88 (3H, s, H-30), 0.99 (3H, s, H-25), 1.11 (3H, s, H-27), 5.33 (1H, br s, H-12), 4.16 (1H, m, H-3e), 4.94 (1H, d, J = 7.6 Hz, H-1'), 6.25 (1H, d, J = 7.8 Hz, H-1''), 6.05 (1H, d, J = 7.9 Hz, H-1'').

$^{13}$C NMR data (100 MHZ, C$_6$D$_5$N) δ 38.4 (C-1, t), 25.7 (C-2, t), 80.7 (C-3, d), 43.0 (C-4, s), 47.3 (C-5, d), 17.6 (C-6, t), 32.3 (C-7, t), 39.1 (C-8, s), 47.6 (C-9, d), 36.3 (C-10, s), 23.0 (C-11, t), 122.0 (C-12, d), 144.2 (C-13, s), 41.5 (C-14, s), 27.7 (C-15, t), 23.2 (C-16, t), 46.0 (C-17, s), 41.1 (C-18, d), 45.8 (C-19, t), 30.3 (C-20, s), 33.6 (C-21, t), 32.6 (C-22, t), 63.5 (C-23, s), 13.5 (C-24, q), 15.5 (C-25, q), 16.8 (C-26, q), 25.5 (C-27, q), 179.0 (C-28, s), 32.7 (C-29, q), 23.1 (C-30, q), 104.1 (C-1', d), 74.7 (C-2', d), 74.6 (C-3', d), 69.1 (C-4', d), 65.6 (C-5', t), 100.5 (C-5''), d), 71.2 (C-6''), d), 78.7 (C-3''', d), 71.8 (C-4''', d), 68.8 (C-5''', d), 18.0 (C-6''', q), 110.3 (C-1''', d), 81.8 (C-2'''', d), 78.2 (C-3''''', d), 87.5 (C-4''''', d), 62.1 (C-5''''', t).

Compound 2

Colourless crystals; [α]$_D$$^{20}$+$^{15.4}$° (c 0.5, EtOH); ESI-HRMS m/z 882.2 ([M-H]$^-$), $^-$ (calc. for [C$_{60}$H$_{58}$O$_{10}$]$_n$ -1, 882.49767400).
\( ^1H \) NMR (400.13 MHz, \( C_6D_6N_2 \)): 1.16 (3H, br s, H-24), 0.95 (3H, s, H-25), 1.05 (3H, s, H-26), 1.57 (3H, s, H-6’’’), 1.25 (3H, s, H-27), 1.00 (3H, s, H-30), 0.95 (1H, d, H-1), 4.90 (1H, d, J = 7.8 Hz, H-1’’’), 6.30 (1H, d, J = 7.8 Hz, H-1’’’), 6.15 (1H, d, J = 7.6 Hz, H-1’’’).

\( ^13C \) NMR data (100 MHz, \( C_6D_6N_2 \)): 8 39.2 (C-1, t), 26.5 (C-2, t), 81.4 (C-3, d), 43.8 (C-4, s), 47.9 (C-5, d), 18.4 (C-6, t), 33.1 (C-7, t), 39.9 (C-8, s), 48.3 (C-9, d), 37.0 (C-10, s), 23.8 (C-11, t), 122.8 (C-12, d), 144.9 (C-13, s), 42.3 (C-14, s), 28.5 (C-15, t), 24.4 (C-16, t), 46.8 (C-17, s), 39.9 (C-18, d), 46.5 (C-19, t), 31.1 (C-20, s), 34.4 (C-21, t), 33.3 (C-22, d), 64.2 (C-23, s), 143.3 (C-24, q), 16.2 (C-25, q), 17.6 (C-26, q), 26.3 (C-27, q), 180.4 (C-28, s), 33.4 (C-29, q), 23.9 (C-30, q), 104.8 (C-1’’, d), 75.2 (C-2’’, d), 74.7 (C-3’’, d), 69.7 (C-4’’, d), 66.4 (C-5’’, t), 101.5 (C-1’’, d), 72.2 (C-2’’, d), 83.1 (C-3’’, d), 73.2 (C-4’’, d), 69.9 (C-5’’, d), 18.6 (C-6’’, q), 107.7 (C-1’’, d), 73.3 (C-2’’, d), 79.9 (C-3’’, d), 69.9 (C-4’’, d), 67.3 (C-5’’, t).

**Acid Hydrolysis of 1 and 2**

Each isolate (10 mg) was heated in a mixture of 20% HCl (6.5 mL) and CH\(_3\)OH (3.5 mL) under reflux for 7 h. The reaction mixture was concentrated under reduced pressure to remove methanol and diluted with water (2 mL) and extracted with chloroform (10 mL ×3). The H\(_2\)O layer was neutralized with Na\(_2\)CO\(_3\), filtered and concentrated under reduced pressure and residue which contained sugars were subjected to paper chromatography analysis with standard sugars glucose, rhamnose, arabinose and xylose. (BuOH-HOAc-H\(_2\)O 4:1:5) was used as solvent and detection was by amiline/phthalate spray.

**Antifungal Assay**

The organisms used in this study were *Candida albicans* (ATCC 90028), *Candida neoformans* (ATCC 90113) and *Aspergillus fumigatus* (ATCC 90906). The antifungal assays were evaluated by agar diffusion method. The antifungal activity was determined by measuring the diameter of zone of inhibition (mm). The determination of MIC was performed using a two fold dilution technique as previously described (Peterson et al., 1992). The MIC, \( \mu \)g mL\(^{-1}\), was recorded as the lowest concentration that prevented visible growth (Hamann et al., 1993). The antifungal agent amphotericin B was included as positive control in each assay.

**RESULTS AND DISCUSSION**

Evidence from \( ^13C \) NMR chemical shifts showed that both 1 and 2, possess the same triterpenoid skeleton, differing only in the sugar regions. A comparison of the \( ^13C \) NMR signals due to the compounds with those of reported saponins revealed that they are monodesmosides of 3-O-glycoside and the aglycones as hederaegenins (Li et al., 1990).

Mineral acid hydrolysis of 1 yielded arabinose and rhamnose as the sugar components. The negative ion HRESI mass spectrum of 1 revealed the molecular ion peaks appearing at m/z 882.3 [M-H]\(^-\) corresponding to the molecular formula \( C_{46}H_{78}O_{36} \) with fragment peaks appeared at m/z 750.9, 604.0 and 471.3 and indicating the loss of arabinose, arabinose + rhamnose and 2-arabinose+ rhamnose. This sequence indicated that a terminal arabinose moiety is linked to an inner rhamnose that is linked to inner arabinose and this, in turn, is attached to the C-3 of hederaegenin (Mahto et al., 1991). This fragmentation pattern confirms interglycosidic linkages in all the sugars of 1. Comparison of \( ^13C \) NMR spectra of the sugar portions with corresponding methylglycosides revealed that the sugar moieties are of \( \alpha-L\)-arabinofuranose, \( \alpha-L\)-arabinopyranose and \( \alpha-L\)-rhamnopyranose (Gorin and Mazurek, 1975).
The $^{13}$C NMR spectrum of 1 demonstrated 46 carbon resonances in partial agreement with the molecular formula $C_{n}H_{2}O_{14}$ indicating three sugar moieties, two pentoses and one hexose while the remaining 30 signals assigned to the aglycone. The $^{13}$C NMR spectrum showed anomeric carbon signals at $\delta_\alpha$ 111.0, 104.9 and 101.3 which were consistent with the presence of trisaccharide chain. The DEPT spectrum displayed 7 methyl, 13 methylene, 18 methane and 8 quaternary carbon atoms. The $^1$H and $^{13}$C NMR and HMBC data indicated the presence of a trisubstituted double bond $\delta_\gamma$ 5.33 ppm (1H, br s, H-12) and $\delta_\gamma$ 14.2 and 122.0 ppm, characteristic of a $\Delta^{11}$ double bond in an oleane skeleton (Ahmad et al., 1993; Silverstein and Webster, 1997). The appearance of signal at $\delta_\gamma$ 179.0 is due to the presence of CO$_2$H group and seven methyl groups: $\delta_\gamma$ 0.99 ppm (3H, s, H-24)/$\delta_\gamma$ 13.5 ppm (C-24), $\delta_\gamma$ 0.80 ppm (3H, s, H-25)/$\delta_\gamma$ 15.5 ppm (C-25), $\delta_\gamma$ 0.88 ppm (3H, s, H-26)/$\delta_\gamma$ 16.8 ppm (C-26), $\delta_\gamma$ 1.46 ppm (3H, s, H-5”)/$\delta_\gamma$ 18.10 ppm (C-5’’), $\delta_\gamma$ 0.88 ppm (3H, s, H-30)/$\delta_\gamma$ 23.1 ppm (C-30), $\delta_\gamma$ 1.11 ppm (3H, s, H-27)/$\delta_\gamma$ 25.5 ppm (C-27) and $\delta_\gamma$ 0.80 ppm (3H, s, H-29)/$\delta\gamma$ 32.7 ppm (C-29).

The points of attachment of the sugar units in 1 were determined from the $^{13}$C NMR chemical shifts (Table 1). The C-3 of the aglycone resonated at $\delta_\gamma$ 80.7, thus showing $+7.0$ ppm deshielding as compared to hederaconin (Li et al., 1990). This is an indication that the sugar moieties are attached at this carbon. The upfield shift of C-2 signal by 1.99 ppm and the $^1$H-HMBC correlation of H-3 ($\delta$ 4.16) to C-1’ ($\delta$ 104.1) also confirmed the above proposed site of glycosidation. The presence of hydroxy group attached to C-23 is evidenced by signal in the downfield region of the $^{13}$C NMR spectrum, $\delta_\gamma$ 63.5 ppm. A comparison of the chemical shift of C-2’ of arabinose (C-2’, $\delta$ 74.7) with that of methyl arabinose (C-2, $\delta$ 71.8) allowed the assignment of a 1→2 linkage between arabinose and rhamnose. The downfield $^{13}$C NMR chemical shift of C-3’’ of rhamnose at 78.7 and small upfield shift of C-4’’ of rhamnose at 71.8 indicated 1→3 linkage between rhamnose and the terminal arabinose. The
nature of the interglycosidic linkage was further confirmed by long-range connectivity information obtained from HMBC spectrum which showed $^{3}$H-HMBC interaction of protons at $\delta$ 4.45 (H-2') and $\delta$ 4.64 (H-3') with anomeric carbons at $\delta$ 100.5 (C-1'') and $\delta$ 110.3 (C-1'') in agreement with presence of (1$\rightarrow$2) and (1$\rightarrow$3) linkages between arabinose and rhamnose and rhamnose and the terminal arabinose, respectively. From these results the structure of 1 was elucidated as 3-O- $\alpha$-L-arabinofuranosyl- (1$\rightarrow$3)-$\alpha$-L-rhamnopyranosyl- (1$\rightarrow$2)-$\alpha$-L-arabinopyranosyl]-hederagenin.

Compound 2 afforded hederagenin as the aglycone and arabinose and rhamnose as sugar components on acid hydrolysis. A comparison of the $^{13}$C NMR spectra of the sugar portions with corresponding methylglycosides suggested that the sugar moieties are of 2 units of $\alpha$-L-arabinopyranose and the other $\alpha$-L-rhamnopyranose (Gorin and Mazurek, 1975) indicating a different sugar composition from 1. The negative ion HRESI mass spectrum of 2 exhibited the molecular ion peak at m/z 882.2 [M-H]$^{-}$ corresponding to the empirical molecular formula $C_{65}H_{93}O_{31}$. The $^{1}$H and $^{13}$C NMR chemical shifts were compared with the literature reports for triterpenoidal sapogenins/saponins (Li et al., 1990), which confirmed the identity of the aglycone as hederagenin. The olefinic resonances at $\delta$ 144.9 and 122.8 corresponding to quaternary and methine behaviour, revealed the presence of unsaturation at C-12 in an oleanane skeleton (Tori et al., 1974). The appearance of a signal at $\delta_{c}$ 180.4 is ascribed to the carbon of CO$_{2}$H group. The DEPT spectrum of 2 displayed 7 methyl, 13 methylene, 18 methine and 8 quaternary carbon atoms. There are seven methyl groups $\delta_{c}$ 1.16 ppm (3H, s, H-24)/$\delta_{c}$ 1.43 ppm (C-24), $\delta_{c}$ 0.95 ppm (3H, s, H-25)/$\delta_{c}$ 16.2 ppm (C-25), $\delta_{c}$ 1.05 ppm (3H, s, H-26)/$\delta_{c}$ 17.6 ppm (C-26), $\delta_{c}$ 1.57 ppm (3H, s, H-6')/$\delta_{c}$ 18.60 ppm (C-6'), $\delta_{c}$ 1.25 ppm (3H, s, H-27)/$\delta_{c}$ 26.3 ppm (C-27), $\delta_{c}$ 1.00 ppm (3H, s, H-30)/$\delta_{c}$ 23.9 ppm (C-30) and $\delta_{c}$ 0.95 ppm (3H, s, H-29)/$\delta_{c}$ 33.4 ppm (C-29). The presence of hydroxy group attached to C-23 was evidenced by signal in the downfield region of the $^{13}$C NMR spectrum, $\delta_{c}$ 64.2 ppm. The anomeric carbon signals resonated at $\delta$ 107.7, 104.8 and 101.5 indicating the presence of three sugar moieties. Mass spectral fragmentation pattern and results of acid hydrolysis tend to suggest identical structure for 1 and 2.
Table 1: $^{13}$C (125 MHz, CD$_3$N) NMR shifts for compounds 1-2

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<th>Position</th>
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<tr>
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The sugar linkages in 2 were determined using the glycosidation rule (Tori et al., 1974; Seo et al., 1978; Mahato et al., 1991). The $^{13}$C NMR spectrum of 2 (Table 1) showed significant displacement of signals for C-3 (+7.7 ppm) of the aglycone, for C-2' (+3.4 ppm) of the arabinopyranosyl moiety and for C-3'' for inner flummopyranosyl moiety (+11.8 ppm) in comparison to the reported values for hederagenin (Tori et al., 1974) and methyl pyranoside due to glycosidation at these positions. The nature of the glycosidic linkage was further confirmed by the HMBC spectrum, which showed 3J-HMBC and 3J-HMBC interaction of protons at δ 4.47 (H-2') and δ 4.63 (H-3), respectively with aromatic carbon C-1'' (δ 104.1) and 3J-HMBC interaction of proton at δ 4.47 (H-2') with C-1'' (δ 100.5). The C-3'' proton at δ 4.64 also showed 3J-HMBC and 3J-HMBC to C-2'' (δ 81.8) and C-1'' (δ 110.3), respectively. However in the $^{13}$C NMR spectrum, different resonance signals were obtained for the terminal α-L-arabinopyranosyl. It was observed that in 2 the signal of C-5'' due to arabinose was displaced downfield by 5.2 ppm and signal C-4''' was displaced upfield by 15.6 ppm. This disclosed that the terminal arabinose has C-5''' attached directly to oxygen to form a pyranose and not a furanose as it was the case with 1. Consequently, 2 was elucidated as 3-O- [α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-hederagenin. Although the two compounds isolated were known compounds (Encarnacion et al., 1981) but the method of isolation and analysis of structures were different.

Compounds 1 and 2 were evaluated for antifungal activity in the agar well- diffusion assay. Compound 1 exhibited antifungal activity against C. albicans, C. neoformans and A. fumigatus with IC$_{50}$ of 4.5 μg mL$^{-1}$, 15.0 μg mL$^{-1}$ and MIC 10.0 μg mL$^{-1}$, respectively and 2 exhibited similar activities with IC$_{50}$ of 8.5 μg mL$^{-1}$, 10.0 μg mL$^{-1}$ and MIC ≥25.0 μg mL$^{-1}$, respectively.

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REFERENCES


