Triterpenoids from *Daniellia oliveri* leaves, Hutch and Dalz (Fabaceae)

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ABSTRACT

Two triterpenoids: a lupane and an oleanane - 9(11), 12- diene acid; were isolated and identified as Lup-20(29)-en-3-ol (Lupenol) and 3-acetoxy -9(11),12-diene-28-carboxyllic acid from dichloromethane extract of the leaves of *Daniellia oliveri*. The structures were elucidated using NMR and MS experiments and compared with data for related compounds.

Key words: *Daniellia oliveri*, triterpenoids, lupane and oleanane.

INTRODUCTION

Daniellia oliveri (Rolfe), Hutch and Dalz (Fabaceae) commonly known as Copaihu africaine, is an indigenous tree found exclusively in Benin, Cameroun, Chad and Nigeria (Dalziel, 1964). The leaves of this plant are used in ethnomedicine in Nigeria to treat diabetes, diarrhea, and gastrointestinal disorder and as diuretic (Hutchinson and Dalziel, 1964; Ahmadu *et al*, 2003). The diterpenes: oliveric and daniellic acid has previously being isolated from the stem bark of this plant, while some flavonoids were isolated from the leaves of this plant (Ahmadu *et al*, 2004a). Antimicrobial, anti-diarrhea and anti-ulcer activities of the leaves of this plant have been reported (Onwukaema and Udoh, 1999; Ahmadu *et al*, 2004b). In this present work, the dichloromethane extract of the air -dried leaves of D. Oliveri was subjected to column chromatography and gel filtration over sephadex led to the isolation of two triterpenoids for the first time from this plant. The structures were elucidated by NMR and MS spectrophotometry.

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MATERIALS AND METHODS

Column chromatography (CC) was carried out using silica gel 230-400 mesh (Fluka): gel filtration was performed using sephadex LH-20 (Sigma chemicals), thin layer chromatography was performed on precoated silica gel F_{254} (0.25 mm), Merck chemicals. NMR spectra were recorded in $CDCl_3$ and TMS as internal standard on a Bruker Avance 600 MHz , 250 MHz spectrophotometer and 150 MHz, 62.5 MHz respectively.

Plant Materials

The leaves of *D. Oliveri* (Leguminasae) were collected at Zaria- Nigeria in July, 2012 and the plant was identified at the Biological Science department, Ahmadu Bello University, Zaria- Nigeria and compared with voucher specimen in the herbarium of the biological science department, Ahmadu Bello University, Zaria.

Extraction

The air -dried and powdered leaves of *D. Oliveri* (300 g) were extracted with dichloromethane (5x500 ml) at room temperature for 24 hours each for seven days. The combined extract was concentrated on a rotary evaporator to give a dark green mass 9.2 g (3.06% w/w). The dried marc was then extracted with 70% methanol (7x500 ml) for seven days to give a brownish mass, 20.9 g (6.97% w/w).

Isolation

Six (6)g of the dichloromethane extract was packed in a column (2 cm x 50 cm) and eluted gradient-wise with n-hexane (100%), n-hexane : ethyl acetate mixtures; 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, ethyl acetate (100%) and finally 5% methanol in ethyl acetate. The progress of elution was monitored with TLC using solvent systems I (n-hexane: ethyl acetate 9:1); solvent system II: (n-hexane: ethyl acetate 5:1) and solvent system III: (2:1). 10 mls aliquot were collected to give a total of 180 fractions. Fractions eluted with n-hexane: ethyl acetate (90:10): (50-70), which revealed similar spots on TLC using solvent system I were pooled together to give 0.11 g. This was purified over sephadex LH-20 eluted with 5% n-hexane in dichloromethane to give compound I, a white amorphous solid 9.2 mg (0.0015 w/w). Fractions eluted with n-hexane: ethyl acetate (80:20), (72-85) which gave three spots were pooled together and purified over sephadex eluted with 5% n-hexane in dichloromethane to give 43 fractions. Fractions (32-39) gave a colourless wax. TLC using solvent system II showed a single spot with R_f value of 0.68.

RESULTS

Compound I, a white amorphous solid (9.2 mg), ^{1}H- NMR ($CDCl_3$): δ = 4.57, 1H (H-29b), 4.69, 1H (H-29a), 3.20 (m), 1H (H-3); 1.70, 3H (s), (H-30); 1.05, 3H (s), (H-27); 0.99, 3H (s) (H-26); 0.97 3H (s) (H-2); 0.85, 3H(s), (H-28); 0.81, 3H (s), (H-25); and 0.78, 3H (s), (H-24)

^{13}C- NMR: (62.5MHz) $CDCl_3$: 38.7(C-1); 27.4 (C-2); 79.0(C-3); 38.8 (C-4); 53.3 (C-5); 18.3 (C-6); 29.3 (C-7); 40.8 (C-8); 50.4 (C-9); 37.2 (C-10); 20.9 (C-11); 25.2 (C-12); 38.7 (C-13); 42.3 (C-14); 27.5 (C-15); 35.6 (C-16); 43.0 (C-17); 48.3 (C-18); 48.0 (C-19); 150.9 (C-20); 29.9 (C-21); 40.0 (C-22); 28.0 (C-23); 15.4 (C-24); 16.1 (C-25); 15.9 (C-26); 14.6 (C-27); 18.0 (C-28); 109.3 (C-29); 19.3 (C-30).

MS(EI-positive mode) m/z = 427 (M^++1) which points to a molecular formula $C_{30}H_{50}O$;

m/z 409, (M^++1 - H_2O)

Compound II, colourless wax

^{1}H-NMR (600 MHz) $CDCl_3$: δ= 5.22, 1H (s,H-12); 5.72, 1H (s, H-11); 2.40,1H (t); 2.20, 3H(s, H-28); 1.60,3H (s,H-23); 1.30, 6H (s,H-25,H-26); 1.0, 3H (s, H-24); 0.90 (H-27); 0.83 (H-29) and 0.78 (H-30).

^{13}C-NMR (125 MHz)$CDCl_3$: 38.4(C-1); 27.5(C-2); 40.4 (C-3); 38.5 (C-4), 46.4 (C-5), 19.9 (C-6), 33.9 (C-7),39.2 (C-8), 164.8 (C-9), 38.7 (C-10), 114.6 (C-11), 120.4 (C-12), 144.5 (C-13), 38.7 (C-14), 27.5 (C-15), 22.7 (C-16), 46.7 (C-17), 38.1 (C-18), 45.8 (C-19), 30.8 (C-20), 33.9 (C-21), 31.9 (C-22), 29.2 (C-23), 16.2 (C-24), 15.5 (C-25), 19.8 (C-26), 24.7 (C-27), 179.6 (C-28), 31.9 (C-29), 22.7 (C-30), 171.6 (COO).

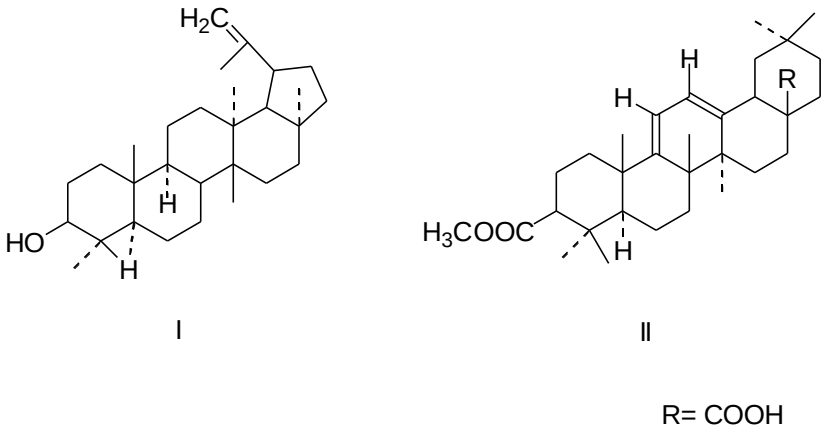

Fig.1 Structures of compounds I and II

DISCUSSION

Chromatographic separation of the dichloromethane extract of *Daniellia oliveri* leaves led to the isolation of two compounds. Compound I, a white amorphous solid appeared to be a lupane-type triterpenoid, as suggested by an intense m/z at 189. The molecular ion peak at m/z (426) (M^++1; 427) corresponded to the molecular formula $C_{30}H_{50}O$. The ^{1}H-NMR spectrum showed seven tertiary methyl singlets at δ 0.78,0.81,0.85,0.97,0.99,1.05 and 1.70, one secondary hydroxyl doublet of doublets at δ at 3.17. It also displayed characteristic proton signal of an isopropenyl group, a down field singlet of vinylic methyl (Me-30) at δ 1.70 and a pair of broad singlets due to exomethylene protons (H-29) at δ 4.55 and 4.67. The double doublets signal at 3.17 is typical for triterpenoid with a 3-hydroxysubstitution. The ^{13}C-NMR of compound I showed 30 signals for terpenoid lupane skeleton which include a carbon bonded to a hydroxyl group at C-3 position which appeared at δ 79.0, while the olefenic carbons of the exocyclic double bond appeared at δ 150.9 and 109.3. The spectral data of compound I were found to be in full agreement with those of lupenol (Reynolds

et al, 1986; Witehnda and Drawan, 2007; Chaturvedula and Prakash, 2012).

Compound II was isolated as a colourless wax. ^{1}H-NMR spectra data revealed seven methyl singlets at δ= 0.78, 0.83, 0.90, 1.0, 1.30 and 1.60; typical of oleanane skeleton (Ragasa and Lim, 2005), except the absence of carbinyl proton in the range 3-3.5 ppm, indicating the absence of 3-hydroxy substitution. Two olefin proton signals at δ = 5.72 and 5.22 assigned to H-11 and H-12 of an oleanane triterpenoid. The position of the two double bonds at $\Delta^{9,11}$ and $\Delta^{12,13}$ were also secured by HMBC correlation of the H-11 (δ = 5.72), with C-10 (38.7) and H-12 (5.22) with C-9 at (δ =164.8) which suggests the presence of a homoannular diene system consistent with that of saikogenin b (Mahato *et al*, 1988). The presence of deshielded carbon signals at δ =171.6 and 179.6 were assigned to carboxylic acid moiety and ester carbon. The positions of these deshielded carbon signals were assigned to C-28 and C-3 of the oleanane skeleton. The HMBC also showed correlation between a deshielded methyl protons at δ = 2.20 ppm with the carbonyl signal at δ= 179.5 which indicates a methyl ester group assigned to C-3. Compound II was found to be 3-acetoxyolean-9(11)-12 dien-28-oic acid. The acetoxy derivative of oleanolic acid 9,(11),13 diene (Saikogenin b); (Lee *et al*,2012).

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