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SHORT COMMUNICATION

Lupane triterpenoid from the stem bark of *Acacia nilotica* (L).Wild.ex.Del (Fabaceae)

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ABSTRACT

From the dichloromethane extract of the stem bark of *Acacia nilotica*, a lupane triterpenoid, lup 20(29)-enol was isolated and the structure elucidated using NMR and MS and is reported here for the first time.

Key words: *Acacia nilotica*, triterpenoid, lupenol

INTRODUCTION

The genus Acacia (Fabaceae) consists of over 1400 species of trees and shrubs widely distributed throughout the warm arid and semi-arid zones of the world which include Nigeria (Predley, 1988, Seigler, 2003). *Acacia nilotica* (L).Wild.ex Del, belongs to the family fabaceae. It is a medium tree 15-18m tall, with a stem diameter of 2-3m having low, spreading and almost symmetrical crown. *Acacia nilotica* has been reported to have a lot of ethnopharmacological uses (Luqman et al, 2015). Among the rich medicinal plants identified so far, *A.nilotica* has been proven to possess remarkable therapeutic benefits against various ailments/diseases such as bacterial,fungal infections and bleeding piles (Luqman et al,2015). The plant has been shown to exhibit anti-hypertensive, anti-cancer activities and is used as a remedy in the relieve of menstrual problem (Ambasta, 1994). The stem bark has been shown to exhibit antioxidant,antimutagenic and cytotoxic activities (Singh *et al*,2008;Singh *et al*,2010; Abbass and El-haq,2015). Flavonols and several galloyl and catechin derivatives have been isolated from the bark of this plant (Khalid et al, 1989), Malan et al, 1991).Previously, we have isolated two new peltogynoids and lupenone from the bark of this plant (Ahmadu et al,2009). In this present work, as part of our continuing study on the phytochemical constituents of *Acacia nilotica*, we report herein the isolation and structure elucidation of a lupane triterpenoid, lupenol from the dichloromethane extract of the stem bark of *A.nilotica*. The structure of this compound was elucidated using spectroscopic technique and compared with literature.

MATERIALS AND METHODS

NMR spectra were recorded on a Bruker 500 MHz and 125MHz spectrometer for ^{1}H and ^{13}C respectively using TMS as internal standard in $CDCl_3$. Mass spectrum was performed on a Thermofinnigan Polaris Q gas chromatography. Mass spectrometer (Direct probe) operating at 50eV. TLC was performed on precoated silica gel TLC plate (0.2mm) aluminium backed (Silicycle), while pressurized column chromatography was carried out on silica gel G (200-400 mesh) Silicycle.

Extraction and Isolation

The bark of *Acacia nilotica* was collected in Zaria in July, 2015. The dried pulverised bark (400g) was extracted to exhaustion with 2.5L of dichloromethane at room temperature and removal of the solvent afforded a brownish mass (1.2 g). A portion of the extract(1.0g) was packed in a column (50cmx3cm) and elution commenced gradiently with n-hexane and dichloromethane ,90:10;80:20;70:30;60:40;50:50;40:60;30:70 ;20:80;10:90 and finally dichloromethane (100%),then 5%methanol in dichloromethane. The progress of elution was monitored on TLC using n-hexane: dichloromethane (1:1) and n-hexane:ethylacetate (9:1). Elution with 60%dichloromethane in n-hexane afforded compound 1, a white solid (7mg).

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RESULTS.

Compound 1, a white solid (7 mg).

^{1}H-NMR ($CDCl_3$),δ: (ppm) 0.74(3H(s),me-24),0.78(3H(s),me-28),0.86(3H(s),me-25),0.92(3H(s),me-27),0.94(3H(s),me-23),1.04(3H(s),me-26),1.7(3H(s),me-30),2.40(IH(m),H-9),3.17(1H(m),H-3),4.54(1H(s),H-29a),4.67(1H(s),H-29b).

^{13}C-NMR ($CDCl_3$),δ:(ppm),150.1(C-20),109.6(C-29),78.5(C-3),55.1(C-5),49.7(C-9),48.2(C-18),47.8(C-19),43.2(C-17),42.6(C-14),41.8(C-8),40.2(C-220,39.2(C-13),38.6(C-4),38.0(C-1),37.3(C-10),35.6(C-16),34.1(C-9),31.0(C-21),28.2(C-23),27.6(C-15),27.5(C-12),25.2(C-2),21.1(C-11),19.5(C-30),18.6(C-6),18.0(C-28),16.8(C-25),16.4(C-26),16.0(C-24),15.1(C-27).

MS: m/z 426 (M^+, 19.2%), 411.3(10.5%), 207.8(100%), 189.2(74%).

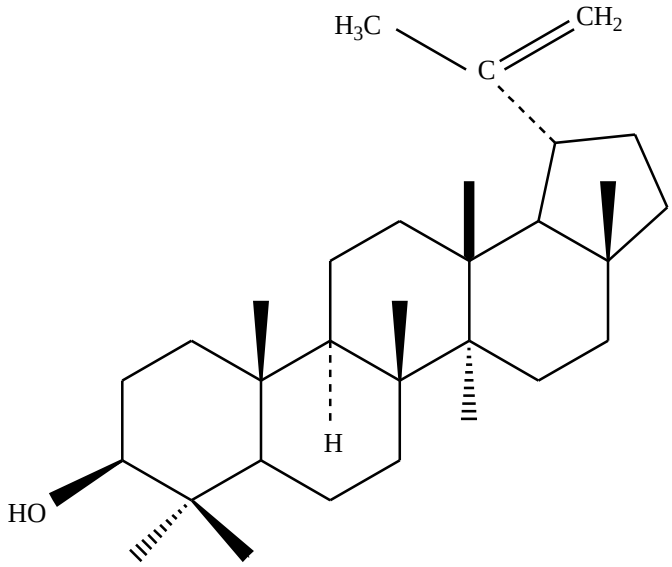

Figure 1: Structure of isolated compound (Lupenol).

DISCUSSION

Compound 1 was isolated as a white solid. Its mass spectral data gave a molecular ion peak m/z at 426 which is consistent with the molar mass of 426 g/mol which points to a molecular formula $C_{30}H_{50}O$.The ^{1}H-NMR spectra revealed seven methyl singlets δ_H 0.74,0.78,0.86,0.92,0.94,1.04 and 1.70 ppm,while a hydroxymethine signal at δ_H3.12 ppm which is typical of 3-hydroxytriterpene derivative was observed. The olefin protons at δ_H 4.54 and 4.67 which is linked to a carbon signal at δ_C109.6, and a low field methyl singlet at δ_C150.1 ppm confirm compound 1 to be a lupane type triterpenoid evident from the isopropenyl side moiety (Baek et al, 2010). The carbon- hydrogen correlation was aided with HSQC-DEPT experiment. The two olefin protons at δ_H 4.55 and 4.67 ppm were linked to the sp2 carbon at δ_C 109.6ppm, while the hyrdoxymethine proton at δ_H 3.17 ppm showed correlation with the carbon signal at δ_C78.5 ppm, the multiplet methine signal at δ_H 2.4 ppm showed correlation with carbon signal at δ_C47.8 ppm. The long range C→H correlation (HMBC experiment) of compound 1, revealed correlation between the exocylic methylene protons (H-29) with the methyl carbon at δ_C19.5 ppm (C-30), correlations were also observed between the exocyclic methylene protons and the methyl protons at C-30 with carbon signal at δ_C 47.8ppm (C-19), long range correlations were also observed between the methyl proton at δ_H 1.70ppm (H-30) with the sp2 carbon at δ_C 109.6ppm and 150.1 ppm. All these correlations confirmed the isopropenyl linkage of compound 1 to C-19 position of the lupane nucleus. Complete assignment of the carbon- hydrogen linkage and their connectivity were aided with the HSQC-DEPT and HMBC experiments.

The mass spectra data of compound gave diagnostic fragments which were consistent with data reported for lupenol(Witchuda and Orawan,2006),such as the molecular ion peak at m/z 426 which is consistent with the molecular formula $C_{30}H_{50}O$. Fragments at m/z 411,218,207 and 189 are typical fragmentation pattern of lupenol (Ogunkoya, 1981). The fragment at m/z 411 is due to M^+-CH_3, m/z 218 is a result of loss of atomic mass unit of 208 from the molar mass (M^+-$C_{14}H_{24}O$) due to retro-Diels-Alders-cleavage of ring C, while m/z 207 (M^+-$C_{16}H_{27}$) loss of mass

unit of 219 from the molar mass, is also due to the retro- Diels- Alders-fragmentation of ring C which is typical of triterpenoids (Ogunkoya,1981), subsequent loss of a water molecule to give the fragment of m/z 189. Compound 1 was found to be lupenol by comparison of the spectral data (NMR and MS) with those reported in literature (Chaturvedula and Prakash, 2012).

CONCLUSION

From the dichloromethane extract, lupenol was isolated for the first time from the bark of *Acacia nilotica*.

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