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Research **Article**

Isolation of Lupeol from the Stem-bark of *Lonchocarpus sericeus* (Papilionaceae)

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Abstract: Lupeol, a pentacyclic triterpenoid, was isolated from the chloroform soluble fraction of a methanol extract of the stem-bark *of Lonchocarpus sericeus,* a plant variously used in ethnomedicine to stimulate appetite, treat constipation, backache and convulsion. Its isolation was carried out by a combination of column chromatography and preparative Thin Layer Chromatography. The structure was determined by analysis of its IR, 'H NMR, ^{13}C NMR, and 2D NMR spectral data, as well as comparison with reported data. This is the first report of isolation of lupeol from the stem-bark of this species.
Keywords: *Lonchocarpus sericeus,* lupeol, NMR spectral analysis

INTRODUCTION

Lonchocarpus sericeus (Papilionaceae), commonly known as Cube root or Senegal lilac is a dry deciduous tree that often grows wild in Nigeria and Ghana and may reach up to 16m high. The main use of the bark in African traditional medicine is as a stomachic and laxative particularly in young children. It also has common application in treating convulsions and back aches [1].

Preliminary phytochemical screening of the methanol extracts of the stem bark revealed the presence of alkaloids, saponins, flavonoids, tannins, triterpenes and steroids [2]. Phytochemical investigation of the root and seed parts led to the isolation of bioactive chalcones, Lonchocarpin and Derricin [3].

The ethnomedicinal use of the stem-bark in the treatment of convulsion was validated scientifically [2]. Despite the medicinal potentials, no study has reported any chemical components of the stem-bark. In this paper, we report the isolation of a pentacyclic triterpenoid of the Lupane group from the chloroform soluble fraction of a methanol extract of the stem-bark of *L. sericeus*.

MATERIALS AND METHODS

Collection and identification of the plant material

The stem-bark of *L. sericeus* was collected from Kongo Campus, Ahmadu Bello University, Zaria, after identification of the tree using description in the literature [1]. The leaves were also collected alongside to ease identification. The identity of the bark was confirmed and authenticated by Messrs Musa Muhammad and U. S. Gallah of the Herbarium Section, Department of Biological Sciences, Ahmadu Bello University, Zaria, through comparison with an existing herbarium specimen of voucher number 1085.

Preparation of the extract

The stem-bark was dried at room temperature under the shade for three weeks and size reduced manually using mortar and pestle. The size reduced stem-bark (2kg) was subjected to extraction with Methanol in a soxhlet apparatus and after evaporation of the solvent, 100g of the extract was obtained. The extract was then suspended in distilled water and filtered. The water insoluble portion was washed with n-Hexane, chloroform and ethyl acetate to yield n-Hexane fraction (nHxF), chloroform fraction (CF) and ethyl acetate fraction (EAF), respectively.

Isolation and purification of compound

A small quantity of CF was dissolved in chloroform and the solution was spotted on TLC plates. The plates were developed using several solvent systems; the solvent systems of Hexane / Chloroform (9:1) and Chloroform / Ethylacetate (5:1) gave better separation of the components, and were used in the TLC monitoring of the Column Chromatography. 10g of the chloroform fraction (CF) was subjected to column chromatography on a silica gel (60 – 120 mesh) with gradient elution using Hexane and Chloroform [4]. Eluents were collected in 25ml aliquots and TLC was used to monitor the fractions.

A total of 104 collections were made and pooled into 9 major fractions, based on their TLC profiles. Fraction 5 indicated significant proportion of the compound of interest and was further subjected to purification by preparative TLC using the solvent system Hexane / Ethylacetate (9:1). A single homogenous spot was obtained on TLC with two different solvent systems Hexane / Ethylacetate (9:1) and (5:1). This compound, coded (R_1), appeared as white needles and was subjected to spectral analysis.

Spectroscopic Characterization

Different spectroscopic methods were used to elucidate the structure of R_1 including IR, ^{1}H NMR, ^{13}C NMR and 2D NMR techniques. The IR spectrum was recorded on FTIR-8400s (Shimadzu) in CCl_4 at NARICT, Zaria; the NMR spectra were recorded on a Bruker AVANCE-300 Japan (100MHz and 400MHz) in deuterated chloroform with TMS as internal standard at the University of Kwazulu Natal, Westville Campus, Durban.

RESULTS AND DISCUSSIONS

The compound (R_1, 6mg), appeared as white needles; mp 120-122°C. IR ν_{max} (CCl_4)cm^{-1}: 3056, 2929, 2313, 1593, 1435, 1265, 898, 741; ^{1}H NMR($CDCl_3$, 400MHz): δ 4.70, 4.55(2H. *s*, H-29a, 29b), 3.2(1H, *m*, H-3), 0.77, 0.79, 0.85, 0.94, 0.97, 1.05, 1.65 (each 3H, *s*); ^{13}C NMR($CDCl_3$, 100MHz): δ 151.0(C-20), 109.0(C-29), 79.0(C-3), 55.5(C-5), 50.5(C-9), 48.3(C-18), 48.0(C-19), 43.0(C-17), 42.9(C-14), 40.9(C-8), 40.0(C-22), 38.9(C-4), 38.7(C-1), 38.1(C-13), 37.2(C-10), 35.5(C-16), 34.2(C-7), 29.9(C-21), 28.0(C-23), 27.4(C-2), 27.1(C-15), 25.2(C-12), 21.0(C-11), 19.5(C-30), 18.5(C-6), 18.0(C-28), 16.1(C-25), 16.0(C-26), 15.5(C-24), 14.8(C-27).

The IR spectrum of R_1 showed characteristic absorption frequencies at 3056 and 1265cm^{-1} typical of the O-H and C-O bond vibrations, respectively; the absorption observed at 898cm^{-1} was due to an unsaturated out of plane C-H vibration; the C=C vibrations was shown around 1593cm^{-1} as weakly intense band; stretching and bending vibrations due to methyl groups were represented by the bands at 2929cm^{-1} and 1593cm^{-1} and the signal at 1435cm^{-1} was due to methylenic vibration.

The ^{1}H NMR spectrum revealed the presence of seven tertiary methyl protons at δ 0.77, 0.79, 0.85, 0.94, 0.97, 1.05 and 1.65 (integrated for 3H-each). A sextet of one proton at δ 2.37 ascribable to 19β –H is characteristic of lupeol. The H-3 proton showed a multiplet at δ 3.2 while a pair of broad singlets at δ 4.55 and δ 4.70 (IH, each) was indicative of olefinic protons at (H-29 a & b). These assignments are in good agreement for the structure of lupeol [5].

The structural assignment of R_1 was further substantiated by the ^{13}C NMR experiments which showed seven methyl groups at [δc: 28.0 (C-23), 18.0 (C-28), 16.1 (C-25), 16.0 (C-26), 15.5 (C-24), 14.8 (C-27) and 19.5 (C-30)]; the signals due to an exomethylene group at [δc: 109.3 (C-29) and 151.0 (C-20)]; ten methylene, five methine and five quaternary carbons were assigned with the aid of DEPT experiment. The deshielded singnal at δc 79.0 was due to C-3 with a hydroxyl group attached to it.

The confirmation of the structure of R_1 was accomplished through the 2D NMR experiments (COSY and HMBC).

The COSY spectrum of R_1 exhibited some cross peaks such as between δ_H 2.37, H-19 and one Sp^3 methylene proton signal (δ_H 1.37, H-21) and another Sp^3methine proton signal (δ H 1.89, H-18); and between oxygenated methine proton signal (δ_H 3.2, H-30 and Sp^3 methylene signal (δ_H 1.60, H-2).

In the HMBC spectrum, the methine proton signal at δ_H 3.2 (H-3) showed cross peaks with a methyl carbon signal (δc 28.0, C-23) by J_2 correlation and a methyl carbon signal (δc 18.5, C-6) by J_3 correlation. The sextet methyl signal at δ_H 2.37 (H-19) showed cross peaks with two methylene carbon signals δc 29.9 (C-21) and δc 109.0 (C-29)], a methine carbon signal [δ_c 48.3 (C- 18), a methyl carbon signal [δ_c 19.5 (C-30)] and a quaternary carbon signal [δ_c 151.0 (C-20)]. The pair of broad singlets of olefinic proton at δ_H 4.55 and 4.70 showed cross peaks with a methylene carbon signal [δ_c 48.0 (C-19) and δ_c 19.5 (C-30)] by J_3 correlation. The forgoing spectral analysis and, comparison with reported data, led us to propose the structure of R1 as lupeol, a pentacylic tri-terpenoid, (figure I) below.

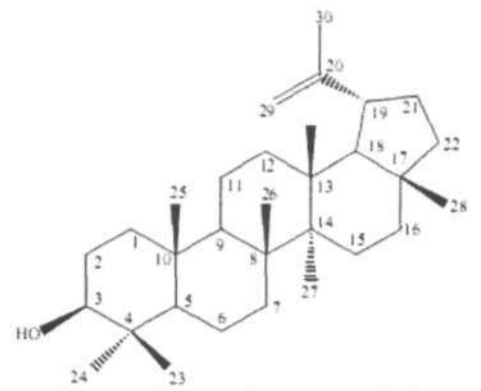


Figure1: Proposed structure of R1: Lupeol

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