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Research Article

Isolation and Characterization of Lup-20(29)-ene-3, 28-diol (Betulin) from the Stem-Bark of *Adenium obesum (Apocynaceae)*

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Abstract

Purpose*: To isolate and characterize chemical compound(s) of biological importance from the stem-bark of the plant, Adenium obesum.*
Methods: *The stem-bark, after air-drying and powdering, was subjected to sequential hot-continuous extraction using petroleum spirit (60 - 80 ^{o}C) and methanol in that order. The petroleum spirit extract was chromatographed using thin layer and column chromatographic techniques. Recrystallization was used to further purify the isolated compound. Characterization of the isolated compound was by melting point, as well as by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).*
Results*: A triterpenoid (lup-20(29)-ene-3, 28-diol), commonly known as betulin, was isolated from the crude petroleum ether extract of the plant stem-bark. The isolated compound's melting point was 256 - 257 °C. The name, betulin, was assigned to this compound by comparison of its spectroscopic data from ^{1}H-NMR, ^{13}C-NMR and MS analysis with those of authenticated samples reported in the literature.*
Conclusion: *A known compound, betulin (lup-20(29)-ene-3, 28-diol) was isolated from the petroleum ether extract of the stem-bark of Adenium obesum.*

Keywords: *Adeniumobesum, Stem bark, Isolation, lup-20(29)-ene-3,28-diol, Betulin*

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INTRODUCTION

Adenium obesum (Apocynaceae) is a small deciduous succulent shrublet that can grow to shrub or small tree that belongs to plant family *Apocynaceae* [1]. The plant, which belongs to the genus *Adenium,* occurs in savanna, dry bushland or woodland and wooded grassland up to 2100 m altitude, on rocky or sandy soil [2]. It is used as poison on arrows [3] and also extensively for the treatment of a variety of ailments including venereal diseases; the root or bark extract is used as a bath or lotion to treat skin diseases and to kill lice, while the latex is applied to decaying teeth and septic wounds to promote healing and restoration. In Somalia, the root decoction, as nasal drops, is prescribed for rhinitis while in northern Kenya, the latex is rubbed on the head to kill lice and the powdered stem is applied on camels and cattle to kill skin parasites. The bark is also chewed as an abortifacient [4]. The ethanol extract of the root has been reported to slow down the growth of *Bacillus subtilis*, while not showing activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albida*. Extracts from the root have been shown to exert cytotoxic effect against several carcinoma cell lines [5, 6].

EXPERIMENTAL

Plant material

The stem-bark of *Adenium obesum* was collected from Samaru area of Zaria; Kaduna State, Nigeria. It was authenticated by Mr. Musa Mohammed of the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where a specimen (voucher number 1836) was deposited. The bark was air-dried and ground into powder using a porcelain, mortar and pestle. It was then sealed in a polyethene bag and stored in a dessicator prior to evaluation.

Extraction

The powdered stem-bark of *Adenium obesum* (500 g) was packed in a thimble, placed inside a soxhlet extractor and extracted exhaustively, first with petroleum spirit (60 - 80 ^{o}C) for 48 h and then with methanol for 42 h. The extracts were concentrated *in vacuo* at 40 ^{o}C in a rotary evaporator to yield the dry extracts.

Isolation of compound

Separation and purification of the various constituents of the crude extract were mainly done by column chromatography (CC) and recrystallization processes. Silica gel 60 (Fluka) was the adsorbent used for the chromatography. The solvent blend used was petroleum ether: ethyl acetate (EtOAC): chloroform in the ratio of 6:2:3. Qualitative thin layer chromatography (TLC) was used to monitor the column fractions and also to ascertain the purity of the isolate. TLC was run on aluminum and glass plates precoated with silica gel 60.F254 (Merck) with a thickness of 0.2 mm each. Visualization of spots on TLC was by Ultra Violet (UV) light and Iodine vapour [7,8]. The melting point of the pure isolate was determined on a Gallenkemp melting point apparatus. Proton (^{1}H) and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Jeol Eclipse spectrophotometer at a frequency of 500 MHz for ^{1}H and 125 MHz for ^{13}C. These spectra were obtained on solutions of samples in deuterated chloroform ($CDCl_3$) with tetramethylsaline (TMS) as internal reference. Mass spectral (MS) were run on a HRFAB mass spectrometer.

RESULTS

The isolate was a white solid with a melting point of 256 - 257 °C. ^{13}C NMR spectrum indicated a total number of thirty carbon atoms, and this number indicates triterpenoidal nucleus. The ^{1}H NMR spectrum six methyl groups at δ 1.67, 0.99, 0.97, 0.96, 0.80 and 0.75 ppm, similar to the

methyls attached to a triterpenoidal nucleus. The molecular ion peak (*m/z* 464.3645) also suggested a triterpenoid.

The results for the proton NMR (1H NMR), 13C NMR and mass spectrometry are presented below:

Spectral data

^{1}H NMR (CDCl3, 500 MHz) *δ* 4.70 (1H, d, H-29b), 4.58 (1H,d, H-29a), 3.79 (1H, d, *J* = 10.8, H-28b), 3.33 (1H, d, *J* = 10.8, H-28a), 3.18 (1H, dd, *J* =5.3, H-3*α*), 1.67 (3H, s, H-30), 0.99 (3H, s, H-27), 0.97 (3H, s, H-26), 0.96 (3H, s, H-23),0.80 (3H, s, H-25), 0.75 (3H, s, H-24).

^{13}C NMR (CDCl3, 125 MHz) *δ* 150.6 (C-20),109.8 (C-29), 79.2 (C-3), 60.6 (C-28), 55.4 (C-5), 50.5 (C-9), 48.8 (C-19), 47.9 (C-17),47.9 (C-18), 42.8 (C-14), 41.0 (C-8), 38.9 (C-1), 38.8 (C-4), 37.4 (C-10), 37.2 (C-13),34.3 (C-7), 34.1 (C-22), 29.8 (C-21), 29.2 (C-16), 28.1 (C-23), 27.5 (C-2), 27.1 (C-15),25.3 (C-12), 20.9 (C-11), 19.2 (C-30), 18.4 (C-6), 16.2 (C-25), 16.1 (C-26), 15.4 (C-24),14.8 (C-27); HRFABMS *m/z* 464.3645

DISCUSSION

A doublet of doublets was present at *δ* 3.17 ppm, which is characteristic of an *α*-oriented hydrogen at C-3 of a 3*β*-hydroxy triterpene. Doublets for geminal protons at *δ* 4.70 and 4.58 ppm, along with the methyl group at *δ* 1.67 ppm, suggests that **1** was a lupeol-type triterpene derivative. Another pair of doublets at *δ* 3.79 and 3.33 ppm, rather than a seventh methyl singlet around *δ* 0.8 ppm, confirms the presence of a second hydroxyl group at C-28. The ^{13}C NMR spectrum further established **1** as a lupeol-type triterpene derivative. The characteristic pair of sp^2 carbons comprising the double bond of lupeol was observed as shifts at *δ* 150.6 and 109.8 ppm [9]. Oxygenated carbon shifts for C-3 and C-28 were observed at *δ* 79.2 and 60.6 ppm, respectively. In all, the spectra revealed a compound with six methyl groups, thirty carbon atoms (which is equivalent to the total number of carbon atoms in triterpenoid), a lupene-type triterpenoidal nucleus with two hydroxyl groups at C-3 and C-28 (a lupeol-type triterpene). Consequently, the compound was determined to be the known structure, 20(29)-lupene-3, 28-diol, more commonly known as betulin (Figure 1). Experimental NMR data was compared to that reported in the literature and all 13C shifts were within ± 0.3 ppm [10,11].

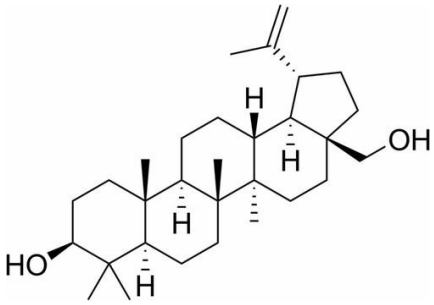

Fig 1: Structure of betulin

CONCLUSION

Based on the investigation conducted on the stem-bark of *Adenium obesum,* a triterpenoid compound, betulin was isolated, characterized and identified as lup-20(29)-ene-3, 28-diol. This compound has been reported to exhibit anti-human immunodeficiency virus (anti-HIV), anti-carcinogenic, anti-flu, anti-inflammatory, immune-omodulator, hepatoprotector, anti-hypoxic, anti-allergen, anti-tuberculosis, anti-tumor, anti-viral, aphidifuge, cytotoxic, hypolipemic, detoxicant (detoxicating agent), adaptogenic and anti-oxidant activities. It also prevents hyperlipidosis and acts as prostaglandin-synthesis and topoisomerase-II-inhibitor [12-15].

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