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A Novel Steroid from Stem Bark of Alstonia Scholaris

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Abstract: **A new steroid, 3-hydroxy adiantulanosterol (5), together with four known compounds, were isolated from the stem bark of *Alstonia scholaris.* All structures were elucidated by spectroscopic methods.**

Keywords: Alstonia scholaris; **steroid; Apocynaceae; 3-hydroxy adiantulanosterol.**

I. INTRODUCTION

Alstonia scholaris belongs to family Apocynaceae, which is widely distributed in tropical eastern Asia, Malayan, Archipelago (Bentham) and subtropical region of the world. In Indian traditional system of medicine, *Alstonia* species are used as medicinal agent [1] for the treatment of various diseases. Genus *Alstonia* is well known for its alkaloidal contents. Various Alkaloids like alstolactone, affinisine oxindole, lagumicine, 10-methoxycathafoline *N* (4)-oxide, alstomaline, 16-hydroxy alstonal, alstophyllal, 6-oxoalstophylline,6-oxoalstophyllal etc. have been reported along with two triterpenes, lupeol acetate and α-amyrin acetate from the title plant [2-3]. It has been reported to show antifertility activity in male albino rats [4]. In our continuing study on chemical constituents from Indian medicinal plants, here within the isolation and structural elucidation of a new steroid, 3-hydroxy adiantulanosterol (5), together with four known compound (1-4) from stem bark of *Alstonia scholaris.*

II. EXPERIMENTAL

A. General Experimental Procedure

Melting points were determined in soft glass capillaries in an electro- thermal melting apparatus. IR spectra were recorded on SHIMADZU FTIR-8400S spectrometer using KBr pellets. ^{1}H NMR and ^{13}C NMR's chemical shift were recorded on JEOL AL-300 spectrometer (300MHz) in parts per million (δ) in CDCl3 with TMS as an internal reference. FAB Mass spectra were recorded on JEOL SX 102/BA-600 mass spectrometer. Column chromatography was performed on silica gel (60-120 Mesh) and TLC on Merck's silica gel 60F254 precoated glass plates.

B. Extraction and Isolation

The stem bark of the plant was collected from university campus, University of Rajasthan, Jaipur (Rajasthan). Shade dried and finely grinded bark (3 kg) was extracted in methanol. Methanol was removed under reduced pressure and the semi-solid mass so obtained was treated with acetonitrile for the removal of fatty part (acetonitrile soluble portion). Acetonitrile was removed under reduced pressure. Fat free extract was again extracted with chloroform and yielded 30 gm of extract after the removal of solvent. The chloroform extract was subjected to column chromatography over silica gel to obtain five fractions. Fraction 1 (pet. ether: benzene, 1:1) contained lupeol acetate as colourless compound, 78 mg, mp 222-224^{o}C. Fraction 2 (benzene, 100%) yielded lupeol as colorless crystals, 57 mg, mp 204-206^{o}C. Fraction 3 (benzene: chloroform, 1:1) afforded α-amyrin acetate, 36 mg, mp 200-202^{o}C. Fraction 4

(benzene: chloroform, 1:3) yielded colorless needles of β-amyrin acetate, 28 mg, mp 216-218°C. Fraction 5 (chloroform, 100%) contained 3-Hydroxy adiantulanosterol as colorless crystals, 26 mg, mp 170-172°C.

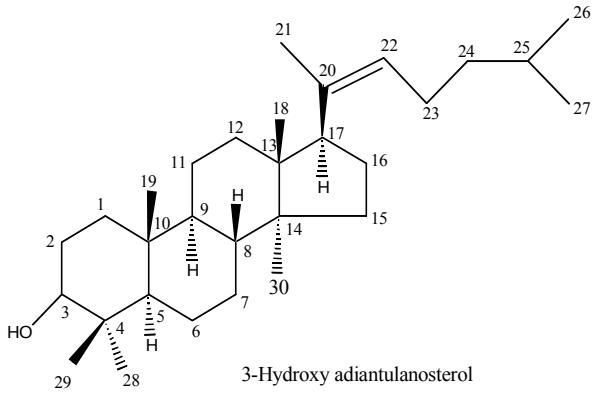

Fig. 1: Structure of novel 3-Hydroxy adiantulanosterol

C. Spectral Data 3-Hydroxy Adiantulanosterol

MP: 170-172°C.

IR (cm^{-1}, KBr): 3650-3440, 2950, 1625, 1610, 1425, 1240, 1080.

^{1}HNMR (δ ppm, 300 MHz, $CDCl_3$): 0.79(3H,s,C-18), 0.89(3H,s,C-19), 1.00 (3H, s, C-28), 1.06(3H,s,C-30), 0.92(3H,s,C-29), 0.86 (3H, d,C-26), 0.82(3H,d,C-27), 1.59(3H,s,C-21), 1.65 (1H,d,C-22), 1.17-1.80 (24 H, C-1 to C- 22).

^{13}CNMR (δ ppm, 300 MHz, $CDCl_3$): 35.5(C-1), 28.5(C-2), 29.7(C-3), 38.1(C-4), 55.6(C-5), 17.2(C-6), 29.8(C-7), 59.4(C-8), 48.0(C-9), 40.0(C-10), 18.6(C-11), 40.4(C-12), 41.4(C-13), 41.9(C-14), 29.6(C-15), 29.7(C-16), 50.4(C-17), 17.2(C-18), 16.1(C-19), 145.49(C-20), 17.9(C-21), 122.93(C-22), 21.8(C-23), 17.9(C-24), 28.5(C-25), 23.3(C-26), 23.7(C-27), 21.8(C-28), 14.5(C-29), 23.1(C-30).

Mass Spectra (m/z): 428.032.

III. RESULTS AND DISCUSSION

3-Hydroxy adiantulanosterol (5) was obtained as colourless crystals. Its molecular formula $C_{30}H_{52}O$, was determined by mass spectrum showing M^+ at m/z 428.032 [M]. The UV, IR, ^{1}H and ^{13}CNMR spectral data of compound 1 were similar to those of compounds isolated from the stem bark of Altonia scholaris [5]. The ^{1}HNMR spectrum of compound (5) showed a singlet proton signal at the range of 0.79-1.59 (ppm) for terminal methyl group of C-18, C-19, C-20, C-28, C-29 and C-30. Doublet signal for C-27, C-26 have been found at δ 0.82 (ppm) which showed free methyl group attatched to a common carbon at C-25. The existence of the upward shift of δ 1.165 (ppm) showed a carbon having double bond. Twenty four protons showed signal at δ range of δ 1.17-1.18 ppm. These all showed a presence of adiantulanosterol skeleton. Signal at δ (ppm) showed a modification of adiantulanosterol into a hydroxyl derivative as (5). Further ^{13}C NMR signal at δ 76.8 ppm showed presence of hydroxyl at position at C-3, and the double bond carbon showed the signal at δ 145.49 (C-20) ppm and δ 122.93 (C-22) ppm. The characterization of lupeol, lupeol acetate, α-amyrin acetate and β-amyrin acetate was done on the basis of mixed m.p.'s and co-TLC with authentic samples and comparision of spectral data with literature [6-11].

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