Alkaloid and Steroid from the Stem Bark of *Jatropha curcas* (Euphorbiaceae)

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ABSTRACT: The plant *Jatropha curcas* grows well in different parts of Bangladesh and used in many medicinal purposes locally. The alkaloid atherospermidine and a steroid stigmasterol were isolated from the ethyl acetate extract of the stem bark of *J. curcas* by a combination of column and preparative thin-layer chromatography over silica gel. The structures of these compounds were determined by spectroscopic analysis (UV, IR, ^{1}H NMR and ^{13}C NMR) and by comparison with published data. This is the first report of isolation of the alkaloid atherospermidine from this plant.

Keywords: *Jatropha curcas*, Alkaloid, Atherospermidine, Stigmasterol, Euphorbiaceae.

INTRODUCTION

Jatropha curcas (Family Euphorbiaceae) is a plant of Latin America which is now wide spread throughout arid and semi arid tropical regions of the world.[1-3] It is as bussy and fast growing plant which grows upto 3-4 m high. This plant is found in every part of the world except very cold areas. Previous phytochemical investigations on different species of *Jatropha* resulted in the isolation of essential oil, sugars.[1,4,5] Among other compounds alkaloids, flavonoids and steroids are most considerable components. In Bangladesh, *J. curcas* is widely distributed in the forest of northern districts. The latex of *Jatropha* contains an alkaloid known as "Jatrophine" which is believed to be anticarcinogenic.[2,6] It is also used an external application for skin diseases and rheumatism and for sores on domestic livestock.[2,7] The tender the treatment of piles.[2,8] The bark of *J. curcas* yields a dark-blue dye which is used in coloring cloth, fishing nets and line. *J. curcas* latex and twigs of the plant are used for cleaning teeth. The juice of the leaf possesses both procoagulant and anticoagulant activities.[3] A number of compounds have been isolated from this plant such as tetradecyl-(E)-ferulate, 3-O-(Z)-coumaroyl oleanolic acid, heudelotinone, epi-isojatrogrossidione, 2-alpha-hydroxy-epi-isojatrogrossidione, and 2-methyanthraquinone.[9] Since this plant has good medicinal properties[2], the present work has been undertaken to isolate and identify biologically active secondary metabolites. In this paper the isolation and structural elucidation of two compounds, the alkaloid; atherospermidine (JC-1) and a steroid stigmasterol (JC-2) are being reported by using spectroscopic techniques like UV, IR, ^{1}H NMR and ^{13}C NMR.

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MATERIALS AND METHODS

General. Melting points were determined on a kolfer hot-stage apparatus and are uncorrected. UV spectrum was taken in MeOH solution using a Perkin-Elmer lambda 9UV/VIS/NIR Spectrometer. IR spectra were recorded in $CHCl_3$ solutions on either a Perkin-Elmer 580 or Philips 9800 FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were

obtained on Bruker WP 200 SY and AM 200 SY instruments (^{1}H, 200. 132 MHz; ^{13}C, 50.32 MHz) using TMS as internal standard and $CDCl_3$ as solvent. Electron impact mass spectra (EIMS) were recorded using a VG updated MS 12 Spectrometer and optical rotations were measured on an optical activity AA-100 Polarimeter in $CHCl_3$ solution at 20ºC. Petroleum ether specifically refers to the bp 40-60^0 fractions.

Plant materials. The stem bark of *J. curcas* was collected from the district of Nilphamari, Bangladesh. The plant has been identified by Prof. Dr. Md. Abul Hasan, Department of Botany, University of Dhaka

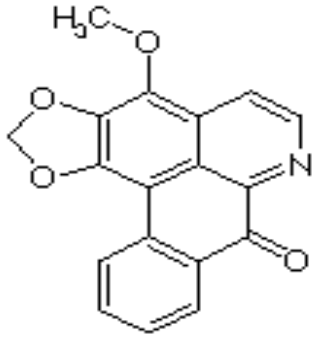
Extraction and isolation. The sun-dried stem bark (950 g) of *J. curcas* was ground and then extracted with petroleum ether, ethyl acetate and methanol sequentially. The cocd. ethyl acetate extract was then mixed with silica gel to prepare paste and the paste was then dried by using a Buchi rotavapor and subjected to flash column chromatography over silica gel (Merck Kieselgel 7-230 mesh). Elution of the column first with petroleum ether, increasing amounts of EtOAc in petroleum ether and finally with methanol yielded a number of fractions. The proportion of solvent systems used to obtain JC-1 (8 mg) and JC-2 (7mg) were petroleum ether: EtoAc (80:20) (90:10) from fractions 14 and 5 respectively.

JC-1 (Atherospermidine) **[1]**, yellowish needles (chloroform); m.p. 282-284°C[10]; UV λ_{max}: 247, 279, 310 nm; IR γ_{max}: 3024, 2380, 1650, 1602, 1518, 1223, 923, 859, 767 cm^{-1}; EIMS m/z (rel. int.): 305 (86), 290 (25), 277 (10), 276 (26), 275 (100), 274 (23), 266 (16), 262 (13), 234 (12), 106 (9), 177 (5), 176 (17), 175 (5), 149 (22); ^{1}H NMR: δ 8.78, 8.09 (2H, Abq, *J*=5.4 Hz, H-4, H-5), 7.65, 7.45 (each 1H, dt, J, 7.6, 1.5 Hz, H-9, H-10) 8.52, 8.46 (each 1H, dt, J=8.0, 0.9, Hz, H-8, H-11) 6.28 (2H, s CH_2O_2), 4.27 (3H, s, CH_3-OCH_3); ^{13}C NMR ($CDCl_3$-OCH_2O): δ 143.8 (C-1), 122.3 (C-1A) 127.4 (C-1B) 135.8 (C-2), 136.8 (C-3), 129.6 (C-3A), 119.6 (C-4), 150.0 (C-6A), 182.4 (C-7), 130.5 (C-7A), 127.3 (C-8), 126.3 (C-9), 134.0 (C-10), 128.0 (C-11), 132.9 (C-11A), 102.3 (CH_2O_2), 59.7 (-OCH_2O-CH_2O_3); HREIMS; Found 305.0672; calculated for C_{18} $H_{11}NO_4$ 305.0688.

RESULTS AND DISCUSSION

Chromatography over silica gel of the ethyl acetate extract of the stem bark of *J. curcas* yielded two compound; an alkaloid, atherospermidine **[**JC-1 **(1)]** and stigmasterol [JC-2]. The structure of these compounds were elucidated by the spectroscopic analysis such as UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy as well as by comparison of their spectral data with the published ones.[10-13]

JC-1 (atherospermidine) **[1]** was obtained as yellowish needles from chloroform. It was UV active and showed absorption at λ_{max} 247, 279, 310 nm. It showed a Dragendorff positive spot on TLC, indicating its alkaloidal nature. The IR spectrum exhibited bands at γ_{max} 1650 (conjugated ketone), 3025, 1602, 1579, and 1518 (aromatic) cm^{-1}. Its mass spectrum revealed a molecular ion peak at m/z 305 corresponding to C_{18} H $_{11}NO_4$, together with fragments at m/z 290 (M^+-CH_3), 277 (M^+-CO), 275 (M^+-CH_2O), 274(M^+-OCH_3), 262 (M^+-CH_3-CO), 149 ($C_{11}H_3N^+$), consistent with an oxoaporphine alkaloid.[10,11]



[1]

The ^{1}H NMR spectrum of JC-1 **[1]** revealed pyridine protons [δ_H 8.09, 8.78 (Abq, J=5.4 Hz,H-4, H-5)], characteristic ring D four spin system with H-8 and H-11deshielded [δ_H 8.46, (1H, dd, *J*=8.0, 0.9 Hz, H-11), 8.52 (1H, dd, *J*=8.0, 0.9 Hz, H-8), 7.65 (1H, dt, *J*=7.6, 1.5 Hz, H-9), 7.45 (1H, dt, *J*=7.6, 1.5 Hz, H-10)], a methylenedioxy group [δ_H 6.28 (s)] and a methoxyl group [δ_H 4.27(s)]. Comparison of ^{1}H NMR

data with the published values.[11,12] confirmed the structure of [**1**] as atherospermidine.

It was clear from these data that JC-1 is closely related to liriodenine but has fully substituted ring A. The ^{1}H NMR data ($CDCl_3$) were consistent with structure (**1**) which is sterospermidine but differ slightly from published values[13] because of the deshielding effects of solvent (TFA). The ^{13}C NMR spectrum is in accord with structure (**1**). It has quaternary for conjugated carbonyl [δ_C182.4], six aromatic methines [δ_C 143.2, 134.0, 128.0, 127.3, 126.3, 119.6], a methylenedioxy group [δ_C 108.3], a methoxy1 groups [δ_C 59.7] and nine aromatic quaternary [δ_C 150.0, 143.8, 136.5, 135.8, 132.9, 130.5, 129.6, 127.4, 122.3] carbons. Comparison with the published data[13] further confirmed the structure of [**1**].

JC-**2** (stigmasterol) was obtained as needle shaped crystals from methanol and has a melting point 160-164ºC. It showed a spot on the TLC plate after spraying with 25% H_2SO_4 and heating. The R_f value of the compound was 0.33 in petroleum ether-ethyl acetate (90:10) on silica gel PF_{254} plate. It was found to be soluble in petroleum ether, methanol, and chloroform. Co-TLC with an authontic sample confirmed the identity of this compound as stigmasterol.

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