

Phytochemical Investigations on the Leaves of *Jatropha curcas*

Momotaz Begum¹, M. H. Sohrab², Farhana Afroz², Shakila Akter², Choudhury M. Hasan³ and A. M. Sarwaruddin Chowdhury¹

¹Department of Applied Chemistry and Chemical Engineering, Dhaka University, Dhaka-1000, Bangladesh

²Bio-assay Laboratories, Analytical Research Division, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh.

³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dhaka University, Dhaka-1000, Bangladesh

Received on 24.11.2009. Accepted for Publication on 30.06.2010

Abstract

Three compounds were isolated from the carbon tetrachloride and n-hexane fractions of the dried leaves from *Jatropha curcas* (Family: Euphorbiaceae). Based on the spectral evidence, their structures were determined to be β -sitosterol-3-O- β -D-glucopyranoside, 7-keto β sitosterol and sitosterol.

Key words: *Jatropha curcas*, Euphorbiaceae, β -sitosterol-3-O- β -D-glucopyranoside, 7-keto β sitosterol, sitosterol.

I. Introduction

Jatropha curcas (Bengali name- Keron gacha, Common name- Bon Beranda, Jamal gota, Shada jeol, Baron, Poison Nut; Family- Euphorbiaceae) is an Erect shrub or small tree up to 6m high young shoots sparingly pubescent. Leaves are light green and slightly palmate. Edges are divided into 4-5 parts, each having sharp points and slightly palmate Flowers are creamy white or pale yellow, small, perianth-5. The origin of *J. curcas* in tropical central and South America and Caribbean islands.² *Jatropha curcas* is now grown in all tropical countries and many sub-tropical regions of the world.

J. curcas is planted around houses, and is used as a therapeutic agent in different ways. Fruits and seeds are used in chronic dysentery, thirst tridosha, urinary discharges abdominal complaints biliousness, anaemia fistula, and diseases of the heart. Twigs are used in tooth brushing when the gums are swollen. The bark of the roots is used as a dressing for sores. The liquor of the leaves is drunk to remove fever. The oil from the seed is applied topically in rheumatism.

Previous phytochemical investigations resulted in the isolation of curcusones A,⁴ cleistasthane,⁵ curcalathyrane,⁵ β -amyrin, taraxerol and stigmasterol³. In this paper, the isolation and structure elucidation of the β -sitosterol-3-O- β -D-glucopyranoside (1), 7-keto β sitosterol (2) and sitosterol (3) by using spectroscopic techniques are being reported.

II. Materials and Methods

General experimental procedure

The ¹H NMR spectra were recorded by using a Bruker DPX-400 (400 MHz) instrument. For NMR studies deuterated chloroform was used and the δ values for ¹H spectra were referenced to the residual nondeuterated solvent signals.

Plant Material The leaves of *Jatropha curcas* were collected from Jessore and identified by, Bangladesh National Herbarium. A voucher specimen has been deposited in Bangladesh National Herbarium (DACB accession no.- 34215) Dhaka, Bangladesh. The leaves were at first sun dried for five consecutive days. Finally the dried leaves were ground into a coarse powder using a grinding machine.

Extraction and Isolation The powdered leaves (500 g) of *J. curcas* was soaked in 2.5 L methanol for 7 days and filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a rotary evaporator. A portion of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method into n-hexane, carbon tetrachloride, dichloromethane and aqueous soluble fractions. The concentrated crude n-hexane fraction (4gm) was subjected to column chromatography for fractionation on silica gel (Kieselgel 60, mesh 70-230) and eluted with gradients of petroleum ether/ethyl acetate, then dichloromethane, after that gradients of dichloromethane/methanol and finally with methanol to afford 28 fractions (each 100 mL). Fraction 25 and 26 washing with petroleum ether, mixture of petroleum ether with dichloromethane gave compound 3 (10 mg) respectively. The concentrated crude carbon tetrachloride fraction (2 gm) was also subjected to column chromatography for fractionation on silica gel (Kieselgel 60, mesh 70-230) and eluted with gradients of petroleum ether/ethyl acetate, then dichloromethane, after that gradients of dichloromethane/methanol and finally with methanol to afford 28 fractions (each 100 mL). Fractions 19, 20, 21 washing with petroleum ether, mixture of petroleum ether with dichloromethane gave compound 2 (10 mg) and fraction 29, 30 washing with petroleum ether, mixture of petroleum ether with dichloromethane gave compounds 1 (15 mg) respectively.

β -sitosterol-3-O- β -D-glucopyranoside (daucosterin)(1)

White powder; ¹H NMR (400 MHz, CDCl₃ + 2 drops CD₃OD) δ 5.33 (1H, brs, H-6), 4.37 (1H, d, J = 8.0 Hz, H-6 β), 3.80 (1H, m, H-5'), 3.75 (1H, m, H-3), 0.88 (3H, d, J = 5.6 Hz, Me-21), 0.84 (3H, s, Me-19), 0.82 (3H, t, J = 7.6 Hz, Me-29), 0.79 (3H, d, J = 7.6 Hz, Me-26), 0.77 (3H, d, J = 8.0 Hz, Me-27), 0.64 (3H, s, Me-18). ¹³C NMR (100 MHz, CDCl₃+2 drops CD₃OD): δ 36.02 (C-1), 29.47(C-2), 79.06(C-3), 39.62(C-4), 122.06(C-5), 138.2 (C-6), 33.81(C-7), 31.80(C-8), 50.04(C-9), 37.12 (C10), 20.92 (C-11), 38.57(C-12), 42.3(C-13), 56.62(C-14), 31.74 (C-15), 29.01(C-16), 55.92(C-17), 11.79(C-18), 19.63(C19), 36.12(C-20), 18.84(C-21), 39.62(C-22), 22.92(C-23), 45.72(C-24), 18.61(C-25), 28.11(C-26), 18.16(C-27),

24.15(C-28), 11.69(C-29), 100.95(C-1'of glucose), 73.40(C-2'), 76.26(C-3'), 69.97(C-4'), 75.60 (C-5'), 61.72(C-6').

7-keto β sitosterol: (2) White powder; ^1H NMR (400 MHz, CDCl_3) δ 3.67 (1H, m, H-3), 5.68 (1H, s, 7-CO), 1.19 (3H, s, 10- CH_3), 0.68 (3H, s, 13- CH_3), 0.92 (3H, d, $J=6.4$, 20- CH_3) 0.82 (3H, d, $J=7.6$, 25- CH_3), 0.83 (3H, d, $J=8.0$, 25- CH_3)

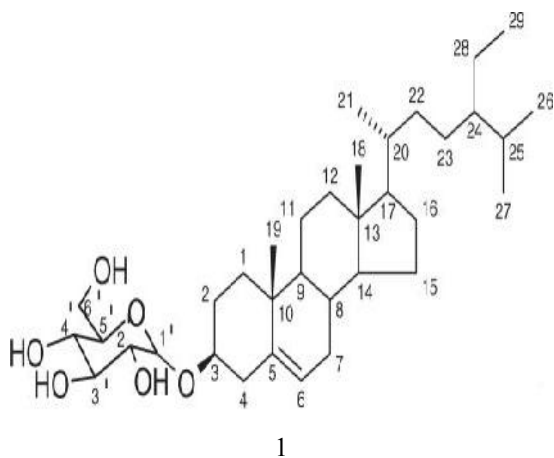
β sitosterol : (3) White crystals; ^1H NMR spectral data was identical to previously reported values.⁷

III. Results and Discussion

β -sitosterol-3-O- β -D-glucopyranoside(daucosterin), 7-keto- β -sitosterol and β sitosterol

were isolated from the carbon tetrachloride and petroleum ether extracts of the leaves of repeated chromatographic separation and purification over silica gel. The structure of the isolated compound was determined by ^1H NMR and ^{13}C NMR data analysis as well as by comparison with previously reported values.

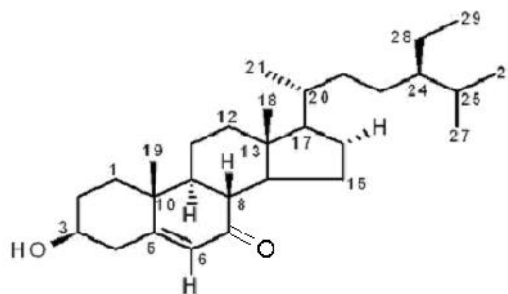
The ^1H NMR spectra (400 MHz, CDCl_3) of compound **1** showed two one-proton multiplets at δ 3.75 and δ 5.33 typical for H-3 and H-6 of a steroidal nucleus. The spectrum further revealed two singlets at δ 0.64 and δ 0.97 each integrating for three protons, assignable to two tertiary methyl groups at C-13 and C-10 respectively. The ^1H NMR spectrum also showed two doublets centered at δ 0.79 ($J = 7.6$ Hz) and 0.77 ($J = 8.0$ Hz) which could be attributed to two methyl group at C-25. The doublet at δ 0.88 ($J = 5.6$ Hz) was demonstrative of a methyl group at C-20. The doublet at δ 4.37 ($J = 8.0$ Hz) showed one proton for H-6 β and another multiplet at δ 3.80 showed one proton for H-5'. The ^{13}C NMR also contained resonances of 35 carbon atoms which were assigned after examination of the DEPT and HMQC Spectra as six methyl groups, 12 methylene, 14 methine and three quaternary



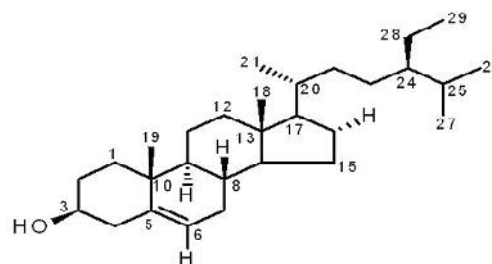
carbon atoms. Particular signals at δ 100.95 (C-1'of glucose), 73.40 (C-2'), 76.26 (C-3'), 69.97 (C-4'), 75.60(C-5') and 61.72 (C-6') indicated the presence of a single

monosaccharide moiety. These signals were in agreement with those obtained from the β -D-Glucose. Finally, the structure of was identified as **β -sitosterol-3-O- β -D-glucopyranoside** by comparing its reported ^1H NMR and ^{13}C NMR data.¹⁰⁻¹²

The ^1H NMR spectrum (400 MHz, CDCl_3) of compound **2** was in close correspondence to that of compound **3**, suggesting a close structural similarity. This spectrum showed one one-proton singlet at δ 5.68 ppm. This significant downfield signal of the olefinic proton compare to the compound **3** (δ 5.68 vs δ 5.33) typical for H-6 of a steroidal nucleus containing a ketone group at C-7 position.



2



3

The ^1H NMR spectrum also showed two one-proton multiplet at δ 3.67 ppm typical for H-3 of a steroidal nucleus. The spectrum further revealed two singlets at δ 0.68 and δ 1.19 each integrating for three protons, assignable to two tertiary methyl groups at C-13 and C-10 respectively. The ^1H NMR spectrum also showed two doublets centered at δ 0.82 ppm ($J = 7.6$ Hz) and δ 0.83 ppm ($J = 8.0$ Hz) which could be attributed to two methyl group at C-25. The doublet at δ 0.92 ppm ($J = 6.4$ Hz) was demonstrative of a methyl group at C-20. These NMR spectral features are characteristics of a steroidal carbon skeleton of β -sitosterol. Finally, the structure of JC-15 was identified as 7-keto- β -sitosterol by comparing its ^1H NMR data to those reported by Morales *et al.*, **2003** and Khastigir H.N.*et al.*, **1976**. Although it is a known natural product, this is the second report of its occurrence from the plant *Jatropha curcus*.

Compound **3** was identified as β sitosterol by comparison of their $^1\text{H NMR}$ spectral data with reported values⁷ as well as by co-TLC with authentic samples

Acknowledgements

The authors wish to thank the Bangladesh Council of Scientific and Industrial Research under Ministry of Science and Information & Communication Technology, Government of the Peoples' Republic of Bangladesh. One of the author's (Momota Begum) thankful to Dhaka University Applied Chemistry and Chemical Engineering Expatriate Alumni for partial funding.

1. Parsons W. and E. Cuthbertson; *Noxious Weeds of Australia.*, 1992, 429–430.
2. Morton JF; A survey of medicinal plants of Curacao. *Econ. Bot.* (1968). 22: 87 – 102.
3. Morton JF; Atlas of medicinal plants of middle America: Bahamas to Yucatan. Charles C. Thomas, Springfield, USA; 1420. (1981).
4. Naengchamnong et al, *J. Chem. Soc.*, 1986, 21, 145
5. Schmeda et al., *Phytochemistry*, 1992, 31, 1731-1735.
6. Khastigir H.N.; B.Saha; D.R.Misra; B.P,Pradhan; *Tetrahedron letter*,1976, **30** ,p 263
7. Morales et al., *Journal of the Chilean Chemical Society*, 2003, **48(2)**. 7-2638
8. Wagenen, B. C. V., Larsen, R. J. H., Cardellina, H. D., Randazzo, Z. C. I. and Swithenbank, C.,1993. *J. Org. Chem.*, **58**:
9. McLughilin, J.L. and Rogers, L.L. 1998. The use of biological assays to evaluate botanicals. *Drug Information* **32**, 513-524.
10. 10. Firouz Matloubi Moghaddam, Mahdi Moridi Farimani, Sabah Salahvarzi, and Gholamreza Amin; Chemical Constituents of Dichloromethane Extract of Cultivated *Satureja khuzistanica*, *Evid Based Complement Alternat Med.* 2007 March; **4(1)**: 95–98.
11. Swift LJ. Isolation of β -sitosyryl-D-glucoside from the juice of flurida *Valencia oranges (Citrus sinensis L.)*. *J Am Chem Soc.* 1952;**74**:1099–100.
12. Escudero J, Lopez C, Rabanal RM, Valverde S. Secondary metabolites from *Satureja* species. New triterpenoid from *Satureja acinos*. *J Nat Prod.* 1985;**48**:128–31.
13. Khan, R. I. (1991) *Natural Product: A laboratory guide*, 2nd Edition, Academic Press, N. Y., USA. Ochse, J.J. 1931. *Vegetables of the Dutch East Indies*. Reprinted 1980. A. Asher & Co., B.V. Amsterdam.
14. Perry, L.M. 1980. *Medicinal plants of east and southeast Asia*. MIT Press, Cambridge.
15. Tewari, J.P. and Shukla, I.K. 1982. Inhibition of infectivity of 2 strains of watermelon mosaic virus by latex of some angiosperms. *Geobios. Jodhpur, India.* 9(3):124–126.
16. Watt, J.M. and Breyer-Brandwijk, M.G. 1962. *The medicinal and poisonous plants of southern and eastern Africa*. 2nd ed. E.&S. Livingstone, Ltd., Edinburgh and London.