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A Novel Bufadienolide from the Seeds of *Mimosa pudica* Linn

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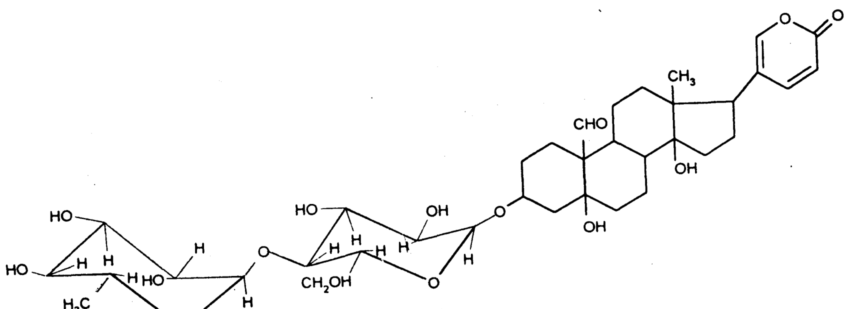
In the present work, we describe the isolation and characterization of a novel bufadienolide from the seeds of *Mimosa pudica* Linn.

INTRODUCTION

Mimosa pudica Linn. (Leguminoseae)[1–3] is commonly known as Lajwanti in Hindi. It is found throughout India. Ayurvedic system of medicine describes that the root of this plant is used in the treatment of biliousness, leprosy, dysentery, asthma, leucoderma, diseases of the blood. The juice of the leaves is useful in dressings or sinus and also as an application for sores and piles. The seeds are used as an effective emetic. The seeds of *Minosa pudica* Linn. were collected from Sagar region and taxonomically authenthicated by the Department of Botany of Dr. Hari Singh Gour Vishwavidyalaya, Sagar (M.P.), India.



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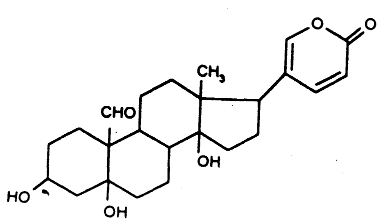

II

EXPERIMENTAL

Air-dried and powdered seeds (2 kg) of the plant were extracted with 90% EtOH and the ethanol soluble fractions was concentrated under reduced pressure to give a brown viscous mass which was further extracted with chloroform. The chloroform extract was concentated and on TLC examination over Si-gel G, it gave two spots which were separated by column chromatography over Si-gel G and eluted with $CHCl_3/CH_3OH$ in various proportions. Eluates collected from fraction (3 : 6) were combined together and found to have same R_f values. It gave compound **I** which was crystallised from methanol to yield light yellow crystals which found to be homogeneous on TLC examination. It gave positive Kedd's reaction[4], Keller Killiani reaction[5] and Legal test[6] indicating its bufadienolide nature. It had m.f. $C_{36}H_{53}O_{15}$, m.p. 284–85°C [found C, 59.61%; H, 7.33%; calcd. C, 59.58%; H, 7.31%]. IR, ν_{max}^{KBr}: 3589, 1783, 1742, 1738, 1721, 1720, 1657 cm^{-1}, ^1H NMR (100 MHz $CDCl_3$): δ 9.99 (1H, s, H-19), 0.71 (3H, s, H-18), δ 4.19 (1H, s, broad, H-3), 7.27 (1H, dd, $J_{21-23} = 1.2$ Hz, $J_{21-22} = 0.8$ Hz, H-21), 7.81 (1H, dd, $J_{22-23} = 9.7$ Hz, $J_{21-22} = 2.9$ Hz, H-22), 6.26 (d, $J_{22-23} = 9.7$ Hz, H-23), 4.75 (1H, d, J = 7.5 Hz, C-1′), 6.52 (1H, d, J = 8 Hz, C-1″). ^{13}C NMR (90 MHz, DMSO-d_6): δ 24.8 (C-1), 27.6 (C-2), 67.4 (C-3), 38.2 (C-4), 75.4 (C-5), 37.2 (C-6), 18.3 (C-7), 42.4 (C-8), 40.6 (C-9), 55.9 (C-10), 22.9 (C-11), 40.5 (C-12), 5.2 (C-13), 85.6 (C-14), 32.5 (C-15), 72.3 (C-16), 51.6 (C-17), 16.8 (C-18), 195.9 (C-19), 177.4 (C-20), 74.6 (C-21), 117.9 (C-22), 176.8 (C-23), 96.1 (C-1′), 120.7 (C-2′), 119.8 (C-3′), 123.9 (C-4′), 117.7 (C-5′), 131.8 (C-6′), 99.2 (C-1″), 121.4 (C-2″), 119.8 (C-3″), 120.9 (C-4″), 175.8 (C-6″); EIMS at m/z: 725, 579, 417.

Acid Hydrolysis of Compound 1: Compound **I** was dissolved in 7% ethanolic H_2SO_4 and refluxed for about 9–10 h. The solution was neutralised with a dilute solution of caustic soda and concentrated under reduced pressure. The aglycone separated out as crystals after drying at 70°C in vaccum. The hydrolysate was neutralized with $BaCO_3$ and $BaCO_4$ was flitered off. The filtrate was concentrated under reduced pressure and subjected to PC examination using n-Butanol acid : Acetic acid : Water (4 : 1 : 5) and aniline hydrogen phthalate as a spraying reagent revealing the presence of D-galactose ($R_f = 0.15$) and L-rhamnose ($R_f = 0.37$).

Study of Aglycone II: It was obtained as light yellow crystals, mp. 282–83°C, m.f. $C_{24}H_{32}O_6$ [found C, 69.25%; H, 7.71%; calcd. C, 69.23% ; H = 7.69%]. IR ν_{max}^{KBr}: 3587, 1785, 1741, 1739, 1722, 1720, 1655 cm^{-1}, ^1H NMR (100 MHz $CDCl_3$), δ 9.97 (1H, s, H-19), 0.71 (3H, s, H-18), 4.16 (1H, s, broad, H-3), 7.26 (1H, dd, $J_{21-23} = 1.2$ Hz, $J_{21-22} = 0.8$ Hz, H-21), 7.79 (1H, dd, $J_{22-23} = 9.7$ Hz, $J_{21-22} = 2.9$ Hz, H-22), 6.24 (d, $J_{22-23} = 9.7$ Hz, H-23).

Periodate Oxidation: Compound **I** was dissolved in MeOH and treated with $NaCO_3$ for 2 days. The liberated 1.05 moles of HCOOH and consumed 3.02 moles of periodate per mole of compound I suggested that both sugars were present in pyranose form.

Permethylation and Hydrolysis of Bufadienolide: Compound **I** was treated with CH_3I and Ag_2O in dimethyl formamide at room temperature for 48 h. The reaction mixture was then hydrolysed with ethanolic H_2SO_4. After usual work methylated sugars were identified as 2,3,6 tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-L-rhamnose which confirmed that C-1′ of D-galactose was attached at C-3 of the aglycone as well as C-4′ of D-galactose was linked with C-1″ of L-rhamnose and (1→4) linkage between D-galactose and L-rhamnose.

RESULTS AND DISSCUSION

The chloroform-soluble fraction of ethanolic extract of the seeds of this plant afforded a novel compound **I** which has the m.f. $C_{36}H_{53}O_{15}$, m.p. 284–85°C, $[M^+]$ 725 (EIMS). Its IR spectrum showed low frequency band at 1720 cm^{-1}, high frequency band at 1742 cm^{-1}, absorption band at 1721 cm^{-1} for C-19 aldehyde group and presence of a pyrone ring at 1785, 1737, 1653 cm^{-1}, ^1H NMR spectrum of I gave signals due to two anomeric protons, one doublet at δ 4.75 (J = 7.5 Hz, D-galactose) and other at δ 6.52 (J = 8 Hz, L-rhamnose). It also showed two double doublets (one proton each) at 7.27 (1H, J_{21-22} = 1.2 Hz, J_{21-22} = 0.8 Hz, H-21) , 7.81 (1H, J_{22-23} = 9.7 Hz, J_{21-22} = 2.9 Hz, H-22) and one doublet at δ 6.26 (1H, d, J = 9.7 Hz, H-23), for C-21, C-22 and C-23 of the pyrone ring. The pyrone side chain was also supported by a mass fragment at m/z 94 and its β-orientation was suggeted[7] by the one proton double doublet at δ 2.74 (J = 9.25 Hz and J = 5.56 Hz) of C-17.

The mass spectrum of **I** gave a (M^+-1) m/z 724, (M^+)m/z 725, a peak at m/z 579 (due to loss of terminal L-rhamnose) and a second peak at m/z 417 (loss of galactose and rhamnose) indicating that C-1′ of the D-galactose was attached at C-3 of the aglycone.

The position of sugar moiety in compound **I** was established by permethylation of **I** followed by acid hydrolysis yielded 2,3,6,-tri-O-methyl-D-galactose and 2,3,4-tri-O-methyl-L-rhamnose according to Petek[8] suggested that the C-1″ of L-rhamnose was linked with C-4′ of D-galactose and C-1′ of D-galactose was attached to the C-3 of the aglycone. The inter linkage (1 → 4) between both sugars were further confirmed by its ^{13}C NMR spectrum (see experimental).

Acid hydrolysis of compound **I** with 7% ethanolic H_2SO_4 yielded aglycone II m.f. $C_{24}H_{32}O_6$, m.p. 282–83°C $[M^+]$ 416. It responded all the characteristic reaction of bufadienolide and identified as hellebrigenin by comparison of its spectral data with authentic sample[9].

The aqueous hydrolysate of compound **I** was neutralized with $BaCO_3$ and $BaSO_4$ filtered off. The filtrate was concentrated and subjected to PC and the sugars were identified as D-galactose (R_f = 0.15) and L-rhamnose (R_f = 0.37). Periodate oxidation of compound I confirmed that both sugars were present in pyranose form[10].

Enzymatic hydrolysis of compound **I** with almond emulsin showed the presence of β-linkage between L-rhamnose and D-galacotse and further on

hydrolysis with Takadiastase suggested the presence of α-linkage between L-rhamnose and aglycone.

On the basis of above discussions, compound **I** was identified as hellebrigenin 3-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-galactopyranoside.

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