Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (5):1607-1611
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Tetrahydroisoquinolic acid derivatives from the seeds of *Mucuna pruriens* Baker

Sunita Singh[a], Suroor Ahmed Khan[a], Mohammed Ali[b]* and K. Ishratullah[c]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India*
[b]*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India*
[c]*Indian Institute of Chemical Technology (CSIR), Uppal Road, Hyderabad-500007, India*

ABSTRACT

A tetrahydroisoquinolic acid glycoside identified as 1,1-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroxyisoquinolin-3-oic acid α-6-β-D-galactopyranosyl-(1→2)-β-D-galactopyrano- side (mucunatetrahydroisoquinolinic acid digalactoside) and n-tetracosan-1, 15 β -diol (mucunatetracosandiol) have been isolated as new phytoconstituents from the seeds of Mucuna pruriens Baker (Papilionaceae) along with the known compounds n-henetriacontane, 1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline-3-oic acid, β-sitosterol and its β-D-glucopyranoside. The structures of all the isolated compounds have been elucidated on the basis of spectral data analyses and chemical reactions.

Keywords: *Mucuna pruriens* Baker; Papilionaceae; seeds; tetrahydoisoquinolinic acids.

INTRODUCTION

Mucuna pruriens Baker; syn. *M. prurita* Hook (Papilionaceae), commonly known as kaunch, atmangupta or velvet bean, is an annual herbaceous, large climber found throughout India among bushes and hedges in damp places and scrub jungles. It is useful as a green manure and cover crop; its pods and young leaves are consumed as vegetable and fodder [1]. In Ayurveda, its seeds are prescribed as an anthelmintic, aphrodisiac, astringent, laxative and tonic, to treat cholera, delirium, impotence, gonorrhoea, leucorrhoea, scorpion sting and to improve vitality [2-4]. The seeds are used to prepare formulations for management of ageing, rheumatoid arthritis, diabetes, male infertility and nervous disorders. The seeds contained fatty oil, β-sitosterol and flavone glycosides [5,6], alkaloids [7], DOPA[8] and N-glycans [9]. The present paper describes the isolation and characterization of a new alkane diol and a tetrahydroisoquinolinic acid along with four known phytoconstituents from the seeds of *M. pruriens*.

MATERIALS AND METHODS

The melting points were determined on a Perfit melting point apparatus and are uncorrected. The IR spectra were recorderd on KBr pellets using Jasco FT-IR-5000 instrument (FTS 135, Hongkong). The UV spectra were scanned in methanol on Lamda Bio 20 spectrophotometer. The ^{1}H NMR (400 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Advance DRX 400, Bruker Spectrospin (Rheinstetten, Germany) in $CDCl_3$ and TMS as an internal

standard. The mass spectra were measured in FAB ionization mode with a JEOL-JMS-DX 303 (Peabody, MA, USA). Silica gel G (60-120 mesh, Qualigens, Mumbai, India) was used for column chromatography. Silica gel G (Qualigens, Mumbai) was used for analytical TLC. Spots were visualized by exposure to iodine vapours, UV radiation and by spraying reagents.

Plant material

The seeds of *M. pruriens* (3 kg) were purchased from Khari Baoli market, Delhi and identified by Prof. M. P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen no. PRL/JH/04/30 is deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction

The air dried defatted seeds (3 kg) were coarsely powdered and extracted with ethanol in a Soxhlet apparatus for 40 hours. The ethanolic extract was concentrated to obtain a dark viscous mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents.

Isolation

The viscous dark brown mass of the ethanol extract was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) to form a slurry. The slurry was air-dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1 and 1:3), pure chloroform and finally mixture of chloroform and methanol (99.5:0.5, 99:1, 49:1, !9:1, 9:1, 4:1, 3:1, 1:1). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same R_f values) were combined and crystallized. The isolated compounds were recrystallized to get the following compounds:

***n*-Henetriacontane (1)**

Elution of column with petroleum ether-chloroform (9:1) afforded colourless amorphous powder of **1;** recrystallized from acetone-methanol (1:1); R_f : 0.23 (n-hexane); 150 mg (o.005% yield); m. p. : 62-64 ^oC ; FAB-MS m/z (rel. int) 436 $[M]^+$ ($C_{31}H_{64}$) (1.3).

Mucunatetracosandiol (2)

Elution of the column with petroleum ether-chloroform (1:1) gave colourless crystalline mass of **2**; recrystallized from methanol. R_f: 0.73 ($CHCl_3$-MeOH, 4:1); 94.3 mg (0.003% yield); m. p. :112-114 ^oC; IR (KBr)ν_{max}: 3417, 3380, 2924, 1607, 1525, 1396, 1296, 1244, 1057, 768 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.76 (1H, m, $w_{1/2}$=16.7 Hz, H-15), 3.10 (2H, m, H_2-1), 1.35 (2 H, m, CH_2), 1.31 (2 H, m, CH_2), 1.29 (2H, m, CH_2), 1.26 (2 H, m, CH_2), 1.23 (32 H, brs, 16xCH_2), 0.82 (3 H, t, J=6.0 Hz, Me-24); ^{13}C NMR (DMSO-d_6): δ 73.12 (C-15), 63.01 (C-1), 45.27 (C-14), 45.05 (C-16), 30.56 (3xCH_2), 29.66 (CH_2), 28.68 (CH_2), 27.27(CH_2), 20.15(CH_2), 14.28 (CH_3-24); FAB-MS m/z : 370 $[M]^+$($C_{24}H_{50}O_2$) (18.9), 257 (21.3), 157 (33.6); HR-FAB-MS: 371.6605 $[M+H]^+$ (Calcd for $C_{24}H_{51}O_2$ 371.6609). Acetylation of **2** (15 mg) with acetic anhydride (5 ml) and pyridine (1 ml) yielded diacetyl derivative, m.p.: 97-98 ^oC and IR ν_{max}: 1733, 1725 cm^{-1}; m/z : 454 $[M]^+$($C_{28}H_{54}O_4$).

Mucunatetrahydroisoquinolinic acid (3)

Elution of the column with chloroform furnished colourless crystalline mass of **3;** recrystallized from methanol. R_f : 0.8 (toluene-ethyl acetate-formic acid; 5:4:1); 125 mg (0.004% yield); m. p.: 180-182 ^oC; UV (MeOH) λ_{max}: 260 nm (log ε-5.7); IR (KBr) ν_{max} : 3310, 3200, 1702, 1509 cm^{-1}; ^1H NMR ($CDCl_3$) : δ 7.23 (1H, dd, J=7.2, 3.0 Hz, H-8), 7.20 (1H, m, H-7), 7.11 (1H, dd, J=7.83, 2.1 Hz, H-5), 7.08 (1H, m, H-6), 3.69 (1H, dd, J=6.9, 7.2 Hz, H-3), 2.45 (1H, d, J=7.2 Hz, H_2-4a), 2.39 (1H, d, J=6.9 Hz, H_2-4b), 0.90 (3 H, brs, Me-10), 0.88 (3H, brs, Me-11); ^{13}C NMR ($CDCl_3$): δ 45.01 (C-1), 44.90 (C-3), 30.14 (C-4), 140.84 (C-4a), 127.25 (C-5), 129.35 (C-6), 129.37 (C-7), 127.36(C-8), 136.94 (C-8a), 180.65 (C-9), 22.37 (C-10), 18.07 (C-11); FAB-MS m/z : 205 $[M]^+$ ($C_{12}H_{15}NO_2$) (22.1).

β-Sitosterol (4)

Elution of the column with chloroform-methanol (49:1) afforded colourless crystalline mass of **4;** recrystallized from methanol. R_f : 0.52 ($CHCl_3$-MeOH, 19:1); 103 mg (0.003% yield); m.p. : 138-139 ^oC**;** (C-29); FAB-MS : 414$[M]^+$ ($C_{29}H_{50}O$) (5.3), 399 $[M-Me]^+$, 396 $[M-H_2O]^+$, 381, 354, 273, 255, 83 and 69.

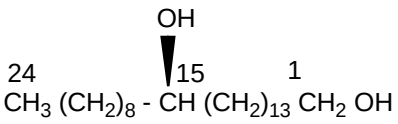
β-Sitosterol-3-β-D-glucopyranoside (5)
Elution of the column with chloroform-methanol (7:3) yielded colourless crystalline mass of **5**; recrystallized from methanol. R_f : 0.54 ($CHCl_3$-MeOH, 4:1); 98.9 mg (0.003% yield); m. p. : 280-282 ^oC; FAB-MS m/z : 577$[M+1]^+$ (6.8), 413 $[M\text{-glucose}]^+$.

Mucunatetrahydroisoquinolinic digalactoside (6)
Elution of the column with chloroform-methanol (3:2) gave brown amorphous mass of **6;** recrystallized from methanol. R_f : 0.66 ($CHCl_3$-MeOH, 1:1); 197 mg (0.006% yield); m. p.: 220-221 ^oC; UV (MeOH) λ_{max}: 246 nm (log 5.6); IR (KBr) ν_{max}: 3366, 3122, 2950, 1698, 1630, 1525, 1391, 1314, 1244, 1069, 807 cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.64 (1H, brs, H-5), 6.56 (1H, brs, H-8), 5.19 (1H, d, J=7.1 Hz, H-1'), 4.95 (1H, d, J=7.0 Hz, H-1"), 4.26 (1H, m, H-5'), 4.19 (1H, m, H-5"), 3.90 (1 H, dd, J = 7.1, 6.5 Hz, H-2'), 3.80 (1 H, dd, J= 7.01, 6.7 Hz, H-2"), 3.77 (1H, m, H-4'), 3.56 (1H, m, H-4"), 3.45 (1H, m, H-3'), 3.41 (1H, m, H-3"), 3.16 (1H, d, J=8.4 Hz, H_2-6'a), 3.11(1H, d, J=8.4Hz, H_2-6'b), 3.00 (2H, brs, H_2-6"), 2.82 (1H, dd, J= 9.5, 5.3 Hz, H-3α), 2.77 (1H, brs, NH-2), 2.52 (1H, d, J= 9.5 Hz, H_2-4β), 2.48 (1H, d, J= 5.3 Hz, H_2-4α), 0.95 (3H, brs, Me-10), 0.91 (3H, brs, Me-11).; ^{13}C NMR (DMSO-d_6) : δ 55.76 (C-1), 51.29 (C-3), 29.45 (C-4), 124.48 (4a), 115.33 (C-5), 144.90 (C-6), 144.42 (C-7), 113.18 (C-8), 123.23 (8a), 180.28 (C-9), 18.28 (C-10), 18.25 (C-11), 104.08 (C-1'), 82.57(C-2'), 69.89(C-3'), 72.87(C-4'), 74.33 (C-5'), 62.19 (C-6'), 91.79(C-1"), 72.33(C-2"), 69.87(C-3"), 70.81(C-4"), 77.15(C-5"), 60.52 (C-6"); FAB-MS m/z : 561$[M]^+$ ($C_{24}H_{35}NO_{14}$) (5.2), 381 $[M\text{-glucose}]^+$(15.3), 220 (17.2), 205(12.5), 175 (11.8); HR-FAB-MS 562.5409$[M+H]^+$ (Calcd for $C_{24}H_{36}NO_{14}$ 562.5411). Compound **6** (25 mg) was heated with 1N HCl (2 ml) in 80% ethanol (5 ml) on a steam bath for 1 hr. After usual work up dihydroxytetrahydroisoquinolic acid, MS 237 $[M]^+$ ($C_{12}H_{15}NO_4$) and D-galactose (co-TLC comparable) were obtained.

RESULTS AND DISCUSSION

Compounds **1**, **3**, **4**, and **5** are the known phytoconstituents identified as n-heneitriacontane, 1,1-dimethyl-1,2,3,4-tetrahydro-isoquinoline-3-oic acid, β-sitosterol [10] and β-sitosterol-3-β-D-glucopyranoside [10], respectively.

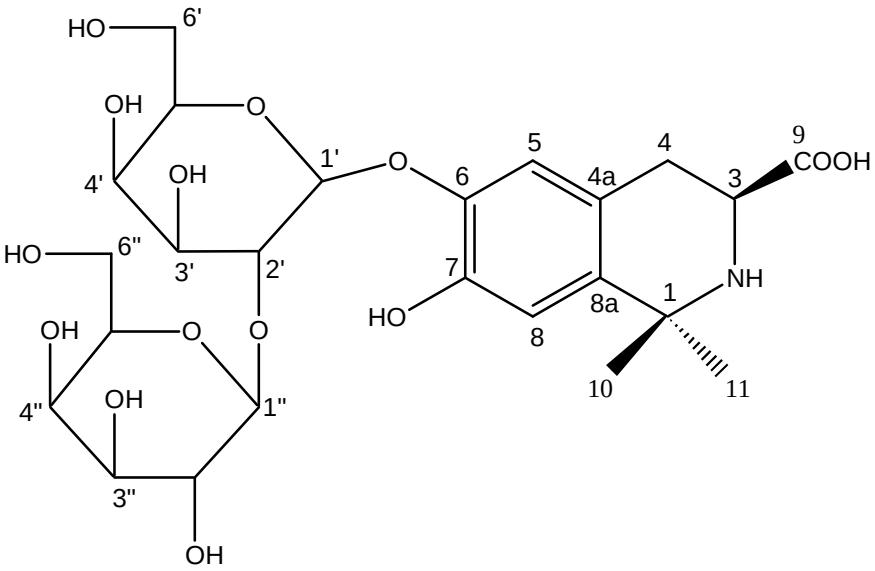
Compounds **2**, named mucunatetracosandiol, was obtained as colourless amorphous mass from petroleum ether-chloroform (1:1) eluents. It did not respond to tetranitromethane and bromine water tests indicating saturated nature of the molecule. It formed acetyl derivative on acetylation suggesting the presence of primary or secondary hydroxyl groups. Its IR spectrum displayed characteristic absorption bands for hydroxyl groups (3417, 3380 cm^{-1}) and for long aliphatic chain (768 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at m/z 370 corresponding to molecular formula of C_{24} aliphatic diol, $C_{24}H_{50}O_2$. The prominent ion fragments at m/z 157 $[CH_3(CH_2)_8CHOH]^+$ and 213 $[M - 157]^+$ aroused due to C_{10}-C_{11} fission supporting the location of the hydroxyl group on C_{15} position. The ^1H NMR spectrum of **2** exhibited a one-proton multiplet δ 3.76 with half width of 16.7 Hz and a two-proton broad signal at δ 3.10 assigned to α-oriented carbinol H-15 and hydroxymethylene H_2-1 protons, respectively. The large coupling interaction of 16.7 Hz suggested α-orientation of the H-15 carbinol proton. The other methylene protons appeared as two-proton each multiplets at δ 1.33, 1.31, 1.29, 1.26 and broad signal at δ 1.23 (32 H). The primary methyl protons Me-24 resonated as a three-proton triplet at δ 0.82 (J=6.0 Hz). The ^{13}C NMR spectrum of **2** displayed signals at δ 73.12 and δ 63.10 for C-15 carbinol and C-1 hydroxymethylene carbons, respectively, methylene carbons from δ 45.27 to 20.15 and primary C-24 methyl carbon at δ 14.28. The absence of any signal beyond δ 3.76 in the ^1H NMR spectrum and δ 73.12 in the ^{13}C NMR spectrum supported the saturated nature of the molecule. Acetylation of **2** yielded diacetyl derivative. Based on these evidences, the structure of **2** was established as n-tetracosan-1, 15 β-diol. This is a new aliphatic diol.



2

Compound **6,** named as mucunatetraisoquinolinic acid digalactoside, was obtained as a brown amorphous powder from chloroform-methanol (3:2) eluants. It gave effervensces with sodium bicarbonate solution and formed green colour with ferric chloride indicating the presence of the carboxylic and phenolic groups in the molecule, respectively. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3366 cm^{-1}), aromatic ring (1630, 1525 cm^{-1}), carboxylic group (1698 cm^{-1}) and secondary amine (3122, 1314 cm^{-1}). On the basis of its FAB mass and ^{13}C NMR spectra the molecular ion peak of 6 was established at m/z 561 consistent with the

molecular formula of a tetrahydroisoquinolic acid diglycoside, $C_{24}H_{35}NO_{14}$. The ion peaks arising at m/z 381 [M-$C_6H_{12}O_6$]$^+$, 220 [M-$C_{12}H_{22}O_{10}$]$^+$, 205 [220-Me]$^+$ and 175 [220-COOH]$^+$ suggested the existence of a diglycoside unit in the molecule. The ^{1}H NMR of **6** displayed two one-proton broad singlets at δ 6.64 and 6.56 assigned to para-coupled aromatic protons H-5 and H-8, respectively. Two one-proton doublets at δ 5.19 (J=7.1 Hz) and 4.95 (J=7.0 Hz) were ascribed to anomeric protons H-1' and H-1", respectively. Other sugar protons resonated between δ 4.26-3.00. A one-proton broad signal at δ 2.77 was assigned to secondary amine protons. A one-proton double doublet at δ 2.82 with coupling interactions of 9.5 and 5.3 Hz was accounted to α-oriented H-3 methine proton. Two one-proton doublets at δ 2.52 (J= 9.5 Hz) and 2.48 (J=5.3 Hz) were due to methylene H_2-4 protons. Methyl protons Me-10 and Me-11 appeared as three-proton broad singlets at δ 0.95 and 0.91, respectively. The ^{13}C NMR of **6** displayed important signals for C-9 carboxylic carbon at δ 180.28, anomeric carbons C-1' at δ 104.08 and C-1" at δ 91.79 and the remaining sugar carbons between δ 82.57-60.52. The aromatic carbons resonated between δ 144.90-113.18. Two methyl carbons C-10 and C-11 appeared at δ 18.28 and 18.25, respectively. The ^{1}H NMR spectrum supported the presence of two β-galactose signals at δ 5.19 (d, J= 7.1 Hz) and 4.95 (d, J= 7.0 Hz). The H-2' was shifted downfield at δ 3.90 indicating that glycosylation of the second galactose unit by the first galactopyranosyl took place on C-2' hydroxyl group. The relatively upfield position of the anomeric proton of one of the galactose unit suggested that it is connected in the molecule of **6** through sugar alcoholic group and not through phenolic hydroxyl. Linkage through the latter would cause these resonances to be shifted relatively downfield [11]. The number and characteristic shifts of ^{13}C sugar signals indicated the presence of two galactose moieties existing in the pyranose form. The presence of C-2' signal in the deshielded region at δ 82.57 in the ^{13}C NMR spectrum supported that position 2' was substituted by the glycosyl chain. The HMBC spectrum of **6** showed correlation of C-9 with H-3 and H_2-4; C-6 with H-5, H-8 and H-1'; and C-2' with H-3', H-1' and H-1". Acid hydrolysis of **6** yielded D-galactose. On the basis of above discussion the structure of **6** has been elucidated 1, 1-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-oic acid-6-β-D-galactopyranosyl-(2→1)-β-D-galactopyranoside. This a new tetrahydroiso-quinolinic acid digalactoside.



6

.**Acknowledgements**
The authors are thankful to the Head, Sophisticated Analytical Instrumentation Facility, Central Drug Research Institute, Lucknow, for recording spectral data of the compounds.

REFERENCES

[1] Anonymous. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, National Institute of Sciences Communication and Information Resources (CSIR), New Delhi, **2003**, Vol. 6, 442-443.

[2] KS Mhaskar; E Blatter; JF Caius. Kirtikar and Basu's, *Illustrated Indian Medicinal Plants*, Sri satguru Publication, Delhi, **2000**, Vol. 3, 1088-1090.
[3] PK Warrier; VPK Nambiar; C Ramankutty. *Indian Medicinal Plants*, Arya vaidya sala, Orient Longman, Hyderabad, **1997**, Vol. 4, pp.-68-72.
[4] JA Parrotta. *Healing plants of India*, CADI Publishing, Oxon, England, **2001**, 404-405.
[5] A Chaterje; SC Pakrashi. *The Tretise on Indian Medicinal Plants*, Publication and Information Directorate, New Delhi, **1992**, Vol. 2, 102-103.
[6] RN Yadav; S Jain. *Asian J. Chem*, **2001**, 13, 1187.
[7] S Ghosal; S Singh; SK Bhattacharya. *Plant Medica*, **1971**, 19, 279.
[8] KP Modi; NM Patel; RK Goyal, *Chem. Pharm. Bull. (Tokyo)*, **2006**, 56, 367.
[9] LDi Patrizi; F Rosati; R Guerranti,; R Paqani; GJ Gerwiq; JP Kamerlinq. *Glycoconj*. **2006**, 23, 599.
[10] L Misra; P Misra; A Pandey; RS Sangwan; NS Sangwan; R Tuli. *Phytochemistry*, **2007**, 69, 1000.
[11] AMD El-Mousallamy; UW Hawas; SAM Husein. *Phytochemistry*, **2000**, 55, 927.