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Alkaloidal constituents of *Mucuna pruriens* seeds

Laxminarain Misra [a,*], Hildebert Wagner [b]

[a] *Phytochemistry Division, Central Institute of Medicinal and Aromatic Plants, P.O. – CIMAP, Lucknow 226 015, India*
[b] *Department of Pharmacy, Center for Pharma Research, Butenandtstr. 5-7, University of Munich, 81377 Munich, Germany*

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Abstract

The seeds of *Mucuna pruriens* (L.) DC. after chemical analysis afforded four tetrahydroisoquinoline alkaloids which have been isolated for the first time from *M. pruriens*. Out of them, two are new whose structures have been elucidated by spectroscopic methods.

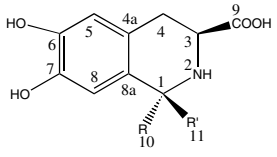
Keywords: *Mucuna pruriens* (L.) DC. syn. *M. prurita* Hk.; Papilionaceae; Seeds; Amino acids; Alkaloids; Tetrahydroisoquinoline; Antiparkinson; Aphrodisiac

1. Introduction

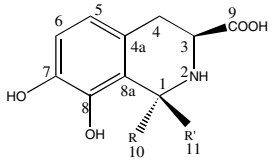
Mucuna pruriens DC. is a herbaceous twining annual found in bushes and hedges at damp places, ravines and scrap jungles throughout the plains of India. It is cultivated for its pods for vegetable and young leaves for fodder. Pods are reported to be used as famine food after repeated boiling and throwing away the water.

Traditionally, in India, the seeds of *M. pruriens* are used as a tonic and aphrodisiac for male virility. The pods are anthelmintic and seeds anti-inflammatory (Anonymous, 1962). The seed powder has recently been found to show the anti-Parkinsonism effects which are probably due to the presence of L-DOPA. It is well known that dopamine is the brain neurotransmitter. The dopamine content in the brain tissue gets reduced because of its blockade of crossing over the blood brain barrier to reach the site of action. As L-DOPA is the precursor of dopamine, it crosses the barrier and gets converted into dopamine resuming the neurotransmission (Kulhalli, 1999).

These important biological actions have led to the chemical investigations of *M. pruriens* seeds to isolate several fatty acids, amino acids besides L-DOPA (Siddhuraju et al., 1996). The presence of alkaloids has also been reported with the tentative names like prurienine, prurieninine, prurienidine, or bases – P,Q,R,S,X etc. (Rakshit and Majumdar, 1956; Ghosal et al., 1971). The structures of these compounds have not been properly elucidated. The present paper describes the presence of 1,2,3,4-tetrahydroisoquinoline alkaloids (**1–4**) whose structures have been determined by spectroscopic methods. It has been shown that 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids (Tic) are very potent and selective towards μ opioid receptors for peptide hormones and neurotransmitters as their bicyclic structure provides conformational constraints and greatly reduced flexibility (Wang and Mosberg, 1955; Kazmierski and Hruby, 1988).



1, R= R'= H
2, R= H, R'= Me
3, R= R'= Me



4, R= R'= Me

* Corresponding author. Tel.: +91 522 2717529; fax: +91 522 2342666.
E-mail address: laxmisra@hotmail.com (L. Misra).

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2. Results and discussion

The spectral data of compounds **1** and **2** were fully comparable to L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and (−)-1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline which have been earlier reported from *M. mutisiana* and *M. deeringiana*, respectively and structure was also confirmed by synthesis (Daxenbichler et al., 1972; Bell et al., 1971). The ^{13}C NMR values of **1** and **2** have also been included in Table 1 as they were not available in the literature and are comparable to the values for similar molecules assigned by Sattoh et al. (2001).

Compound **3**, assigned the molecular formula $C_{12}H_{15}O_4N$ on the basis of HREIMS which had a difference of 14 in $[M]^+$ compared to **2**. The ^{1}H NMR (Daxenbichler, 1972) spectrum of **3** was very much similar to **2** except having two signals for methyls at δ 1.61 and 1.75. Similarly, the ^{13}C NMR compared well with that of **2** except for the two methyl signals at δ 28.7 and 27.9. The other signals in ^{1}H NMR were the typical ABX system at δ 3.19, 3.02 and 3.93 (J = 6, 11, and 16 Hz). Also, the two singlets at δ 6.59 and 6.71 in ^{1}H NMR clearly supported the presence of isolated protons at C-1 and C-4 in the benzene ring. The NOE experiments showed strong effect of H-10 upon H-3 and weak effect of H-11 and H-4 upon H-8 and H-5, respectively. Similarly, the values of ^{13}C NMR (Table 1) and IR established the 3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline structure with dimethyl at C-1 for compound **3**.

Compound **4** also showed a similar ^{1}H NMR spectrum to **3** except for the presence of two doublets at δ 6.55 and 6.77 (J = 8.0 Hz) in place of two singlets at δ 6.59 and 6.71 in **3**. This difference clearly indicated that these protons are located at ortho position to each other, substantiating the fact that the hydroxyl groups are situated either at 5 and 6 or 7 and 8 positions. Since the NOE experiment on methyls at δ 1.97 and 1.78 did not show any effect on these doublets, it was obvious that the hydroxyls are positioned at 7 and 8 in this compound. However, the NOE showed strong effect upon H-3 and weak effect on H-5. The rest of the spectral data (^{13}C NMR, HRMS and IR) were in complete agreement with the structure of **4** as (−)-3-carboxy-1,1-dimethyl-7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline.

The biogenesis of tetrahydroisoquinoline alkaloids has been studied by several groups and there exists conflicting ideas about the origin of C-1 in this type of nucleus (Kapadia et al., 1970; Herbert and Mann, 1982). Now, with the isolation of non-, mono-, and di-methyls at C-1 from a single plant, it is evident that during the biosynthesis of such molecules, a dimethyl precursor, in addition to acetaldehyde/α-keto acids, may also condense with the 3,4-dihydroxyphenethylamine to form compounds like **3** and **4**.

Table 1
^{13}C NMR spectral data for compounds **1**–**4** (CD_3OD, 100 MHz)

Carbon	1	2	3	4
C-1	55.2 (CH_2)	56.7 (CH)	58.9 (C)	59.5 (C)
C-3	54.2 (CH)	52.0 (CH)	54.0 (CH)	53.8 (CH)
C-4	30.5 (CH_2)	28.9 (CH_2)	30.6 (CH_2)	31.1 (CH_2)
C-4a	123.4 (C)	123.9 (C)	122.7 (C)	124.4 (C)
C-5	113.3$^{\gamma}$ (CH)	112.9$^{\gamma}$ (CH)	112.3$^{\gamma}$ (CH)	120.3$^{\delta}$ (CH)
C-6	146.0$^{\delta}$ (C)	143.4$^{\delta}$ (C)	145.7$^{\delta}$ (C)	115.3$^{\delta}$ (CH)
C-7	145.2$^{\delta}$ (C)	144.0$^{\delta}$ (C)	146.0$^{\delta}$ (C)	144.4$^{\gamma}$ (C)
C-8	115.7$^{\gamma}$ (CH)	115.8$^{\gamma}$ (CH)	115.9$^{\gamma}$ (CH)	143.1$^{\gamma}$ (C)
C-8a	124.4 (C)	125.1 (C)	129.7 (C)	123.3 (C)
C-9	178.5 (C)	178.9 (C)	179.0 (C)	179.3 (C)
C-10	–	17.8 (CH_3)	28.7* (CH_3)	26.1* (CH_3)
C-11	–	–	27.9* (CH_3)	24.1* (CH_3)

$^{\gamma,\delta,*}$ Values interchangeable for substance.

3. Experimental

3.1. General

NMR spectra were recorded on a 400 MHz JEOL and IR on a Perkin–Elmer 1710 instruments. The HREIMS were recorded on Jeol-JMS 700 while optical rotations measured on Horiba SEPA 300. MPLC was carried out on Gilson instrument model 303, 500 psi, flow rate 2.5 ml/min in Buechi glass column on si gel 0.015–0.040 mm with mobile phase as butanol–propanol–water, 6:6:1.

3.2. Plant material

The commonly available seeds of *M. pruriens* were bought from the local market of Lucknow, India in November 1999 and the identification of the material was verified by the taxonomists of CIMAP and the voucher specimen was deposited in the Department of Pharmacy of the University of Munich.

3.3. Extraction and isolation of compounds

The dried, milled seeds (500 g) were defatted with acetone (1.25 l) by shaking for 48 h at RT. The defatted material was extracted with *n*-propanol (3 × 1.25 l) by shaking over night. Filtrate were pooled together and concentrated. 1/3 of the above extract after MPLC on si gel 0.015–0.040 mm with butanol–propanol–water, 6:6:1 gave 7 main fractions. Fr. 1 gave a mixture of fatty acids, fr. 2 gave a complex mixture which after TLC (butanol–propanol–water–acetic acid, 3:3:1:1) gave **1** (8 mg, R_f 0.80), **3** (20 mg, R_f 0.75), **4** (12 mg, R_f 0.60, fr. 3 after TLC (butanol–propanol–water–acetic acid, 3:3:1:1) afforded **3** (25 mg, R_f 0.75), **4** (12 mg, R_f 0.60), **2** (7 mg, R_f 0.50), fr. 4 gave a complex mixture of above mentioned alkaloids, fr. 5 after MPLC (system as described above)

and TLC (butanol–propanol–water–acetic acid, 3:3:1:1) gave **2** (10 mg), fr. 6 after crystallization afforded L-DOPA (30 mg) and fr. 7 gave a mixture of amino acids.

*3.3.1. (−)3-Carboxy-1,1-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**3**)*

Crystallizable viscous mass; $[\alpha]_D^{35}$: −155.3° MeOH, $c = 0.07$); IR ($CHCl_3$) cm^{-1}: 3400 (OH), 3340 (NH), 1622 (CO), 1370, 1360 (gem-dimethyl). HREIMS (70 eV) *m/z* (rel. int.): 237.2572 (10) (Calc. For $C_{12}H_{15}O_4N$ 237.255), 222 (5) $[M–Me]^+$, 207 (25) $[222–Me]^+$, 193 (27) $[M–CO_2]^+$, 175 (8) $[193–H_2O]^+$, 157 (8), 133 (100), 127 (28), 107 (88); ^1H NMR spectral data (400 MHz, CD_3OD) δH: 1.61 and 1.74 (3H each, *s*, Me), 3.93 (1H, *dd* ($J = 11$, 6 Hz), H-3), 3.19 (1H, *dd* ($J = 16$, 6 Hz), H-4), 3.02 (1H, *dd* ($J = 16$, 11 Hz), H-4′, 6.59 (1H, *s*, H-5), 6.71 (1H, *s*, H-8); NOE, % (CD_3OD): H-10 → H-3 (8), H-11 → H-8 (3), H-4 → H-5 (3); ^{13}C NMR spectral data (100 MHz, CD_3OD) Table 1.

*3.3.2. (−)3-Carboxy-1,1-dimethyl-7,8-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**4**)*

Crystallizable viscous mass; $[\alpha]_D^{35}$: −144.2° MeOH, $c = 0.01$); IR ($CHCl_3$) cm^{-1}: 3400 (OH), 3345 (NH), 1628 (CO), 1370, 1360 (gem-dimethyl). HREIMS (70 eV) *m/z* (rel. int.): 237.2575 (5) (Calc. For $C_{12}H_{15}O_4N$ 237.255), 222 (2) $[M–Me]^+$, 207 (3) $[222–Me]^+$, 193 (53) $[M–CO_2]^+$, 175 (7) $[193-H_2O]^+$, 157 (3), 127 (19), 125 (100), 117 (63), 107 (58); ^1H NMR spectral data (400 MHz, CD_3OD) δH: 1.97 and 1.78 (3H each, *s*, Me), 3.97 (1H, *dd* ($J = 11$, 6 Hz), H-3), 3.21 (1H, *dd* ($J = 16$, 6 Hz), H-4), 3.07 (1H, *dd* ($J = 16$, 11 Hz), H-4′, 6.77 (1H, *d* ($J = 8$ Hz), H-5), 6. 55 (1H, *d* ($J = 8$ Hz), H-6); NOE, % (CD_3OD): H-10 → H-3 (10), H-4 → H-5 (2); ^{13}C NMR spectral data (100 MHz, CD_3 OD) Table 1.

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References

Anonymous, 1962. The Wealth of India, PID, CSIR, New Delhi, pp. 439–444.

Bell, E.A., Nulu, J.R., Cone, C., 1971. L-DOPA and L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline: a new imino acid from seeds of *Mucuna mutisiana*. Phytochemistry 10, 2191–2194.

Daxenbichler, M.E., Kleiman, R., Weisleder, D., VanEtten, C.H., Carlson, K.D., 1972. A new amino acid, (−)-1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline from Velvet beans. Tetrahedron Lett. 18, 1801–1802.

Ghosal, S., Singh, S., Bhattacharya, S.K., 1971. Alkaloids of *Mucuna pruriens* – chemistry and pharmacology. Planta Med. 19, 279–284.

Herbert, R.B., Mann, J., 1982. The biosynthesis of β-carboline alkaloids, Harman and Eleagnine. J. Chem. Soc., Perk. Trans. I, 1523–1525.

Kapadia, G.J., Subba Rao, G., Leete, E., Fayer, M.B.E., Vaishnav, Y.N., Fales, H.M., 1970. On the origin of carbon 1 in tetrahydroisoquinoline alkaloids. J. Am. Chem. Soc. 92, 6943–6951.

Kazmierski, W., Hruby, V.J., 1988. A new approach to receptor ligand design: synthesis and conformation of a new class of potent and highly selective μ opioid antagonists utilizing tetrahydroisoquinoline carboxylic acid. Tetrahedron 44, 697–710.

Kulhalli, P., 1999. Parkinson's disease therapy – an overview. Heritage Heal. July, 29–30.

Rakshit, S., Majumdar, D.N., 1956. *Mucuna pruriens* DC. Part V. Alkaloidal constituents and their characterization. Indian J. Pharm. 18, 285–287.

Sattoh, T., Ichikawa, T., Horiguchi, T., Toda, J., Sano, T., 2001. A synthesis of 3-phenyl-1,2,3,4-tetrahydroisoquinoline and 2-phenyl-1,2,4,5-tetrahydro-3H-3-benzazepine via Pummerer-type cyclization: enhancing effect of boron trifluoride diethyl etherate on the cyclization. Chem. Pharm. Bull. 49, 979–984.

Siddhuraju, P., Vijayakumari, K., Janardhanan, K., 1996. Chemical composition and protein quality of the little known legume, velvet bean (*Mucuna pruriens* (L.) DC.). J. Agri. Food Chem. 44, 2636–2641.

Wang, C., Mosberg, H.I., 1955. Synthesis of a novel series of topographically constrained amino acids: benzo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids. Tetrahedron Lett. 36, 3623–3626.