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# Some compounds contained nitrogen and steroids from the seeds of *Mucuna pruriens*

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***Abstract:***

***Mucuna pruriens* was used in traditional medicine by many nations on earth. It has possessed many bioactivities, especially as seeds. Up to now, there have not been many publications about its chemical constituents, both pharmacological and clinical, in Vietnam. In our research, a phytochemical investigation of the ethyl acetate and aqueous extracts from the seeds of *M. pruriens* led to the identification of three nitrogenous compounds: *L*-dopa (1), 5,6-dihydroxyindole (2), and *L*-proline (3), as well as two known compounds, stigmasterol (4), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (5). The compounds were characterised using utilisation of electrospray ionisation - mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) spectroscopy, and the results were compared with previously reported data. Compounds 2 and 3 have been isolated from this plant for the first time. In addition, the main constituent was identified as *L*-dopa (1), which has been shown to be a significant marker for chemotaxonomy. This chemical data will contribute new knowledge of this plant in Vietnam and around the world. Furthermore, our research findings can be applied in-depth studies of clinical medicine and pharmacology in the future.**

***Keywords:*** ***L*-dopa, *L*-proline, *Mucuna pruriens*, 5,6-dihydroxyindole.**

***Classification numbers:*** **3.3, 3.5**

## 1. Introduction

*Mucuna pruriens* (L.) DC commonly known as velvet bean [1], belongs to the Fabaceae family and is widely distributed in equatorial and sub-equatorial regions, including Asia, Africa, the Pacific Islands, tropical America, the West Indies, and the USA [2]. *M. pruriens* is a traditional herb renowned for its numerous bioactivities, such as antimicrobial, anti-diabetic, anti-inflammatory, antioxidant, antineoplastic, antiepileptic, and anti-Parkinson effects [1, 2]. Numerous previous studies have shown that the primary chemical constituents of the plant are alkaloids, such as 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolone, 5-hydroxytryptamine, 5-oxyindole-3-alkylamine, and 6-methoxyharman [3, 4], along with amino acids such as isoleucine, lysine, and glutamic acid [5], particularly *L*-dopa. *L*-dopa, another name for levodopa, has the ability to cross the blood-brain barrier, unlike dopamine [6]. For this reason, *L*-dopa is used to increase dopamine concentrations in the treatment of Parkinson's disease. In our studies using methanol extracts from this plant's seeds, five compounds, including *L*-dopa (**1**), 5,6-dihydroxyindole (**2**), *L*-proline (**3**), stigmasterol (**4**), and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**5**), were isolated and structurally confirmed.

## 2. Materials and methods

### *2.1. General experimental procedures*

Thin-layer chromatography (TLC) stains were visualised under UV illumination (254 and 365 nm), followed by dipping in a 10% (v:v) aqueous solution of $H_2SO_4$ or a 1.7% ninhydrin solution and subsequent heating. Column chromatography (CC) was conducted using adsorption materials such as silica gel (particle size 0.063-0.200 mm, Merck, 70-230 mesh), reverse phase powder RP-18 (ODS-A, 75 mm, YMC, Japan), and Diaion HP-20 resin (0.25-0.85 mm, Mitsubishi Chemical Corp., Japan). Optical rotations were measured with a JASCO P-2000 polarimeter. NMR spectral data were obtained using a Bruker AM600

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FT-NMR spectrometer (Bruker, Rheinstetten, Germany). High-resolution electrospray ionisation mass spectrometry (HR-ESI-MS/ESI-MS) was performed with a waters quadrupole time-of-flight (QTOF) micro mass spectrometer.

### *2.2. Plant materials*

*Mucuna pruriens* seeds were collected from Son Dong district, Bac Giang province, Vietnam, in September 2022 and identified by Dang Minh Tu and Nguyen Quynh Nga at the Centre for Medicinal Plant Resources, National Institute of Medicinal Materials. The voucher specimens (code: NIMM-19206) were deposited there.

### *2.3. Extraction and isolation*

The powdered seeds of *M. pruriens* (4.0 kg) were drawn out by methanol at room temperature (3 times, 3×12 litres). After concentrating under decreased pressure to give 400.2 g of extract which was dissolved in 1.000 ml and then sequentially apportioned with *n*-hexane and ethyl acetate, respectively. Vacuum vaporisation of the organic solvent produced the corresponding extracts (19.65 and 85.75 g, respectively). Three fractions were obtained by loading the water layer onto the Diaion HP-20 column and rinsing it with a solvent system MeOH:$H_2O$ (0:100 to 100:0 v:v) MPW1 (274.57 g), MPW2 (15.2 g), and MPW3 (5.03 g). The ethyl acetate extract was subjected to silica gel column chromatography and eluted with a solvent system of $CH_2Cl_2$:$CH_3OH$ (100:0 to 0:100; v:v) to yield 43 fractions (MPE1-MPE43). Mixed fractions (MPE13-MPE17) were further separated using a silica gel column and eluted with *n*-hexane:ethyl acetate (4:1, v:v) to give fraction MPE7.1. The known alkaloid 5,6-dihydroxyindole (5.4 mg) (**2**) was obtained by further purifying fraction MPE7.1 using column chromatography on silica gel, eluting with *n*-hexane-EtOAc (4:1, v:v). Fractions MPE21-MPE24 were crystallised and washed with cold methanol to yield stigmasterol (100 mg) (**4**). Additional fractions (MPE41-MPE42) were crystallised and washed with cold methanol to yield *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (30 mg) (**5**). Nine fractions (MPW11.1-MPW11.9) were obtained by silica gel chromatography, eluting with *n*-$C_4H_9OH$:$H_2O$:$CH_3COOH$ (6:1:1, v:v:v) from fraction MPW2 (15.2 g). *L*-dopa (11.9 mg) (**1**) was obtained by further purifying fraction MPW11.4 using a Sephadex LH-20 column, eluting with MeOH:$H_2O$ (1:1, v:v). *L*-proline (6.8 mg) (**3**) was obtained from fraction MPW11.7 by Sephadex LH-20 column desorption with MeOH:$H_2O$ (1:1, v:v).

*L*-dopa (**1**): White powder; melting point: 276°C; $[\alpha]^{20}_D$ -11.7° (c = 5.3; 1N HCl; molecular formula $C_9H_{11}NO_4$; ESI-MS (*m/z* 197.8) $[M+H]^+$; $^1$H NMR (600 MHz, $CD_3OD$); $^{13}$C NMR (125 MHz, $CD_3OD$).

5,6-dihydroxyindole (**2**): White powder ; melting point: 140°C; molecular formula $C_8H_7NO_2$; HRESI-MS (*m/z* 150.0251) $[M+H]^+$; $^1$H NMR (600 MHz, $CD_3OD$); $^{13}$C NMR (125 MHz, $CD_3OD$).

*L*-proline (**3**): White powder; melting point: 228°C; $[\alpha]^{20}_D$ -85.5°(c =4. $H_2O$); molecular formula $C_5H_9NO_2$; ESI-MS (*m/z* 115.9) $[M+H]^+$; $^1$H NMR (600 MHz, $CD_3OD$); $^{13}$C NMR (125 MHz, $CD_3OD$).

Stigmasterol (**4**): White powder; melting point: 160°C; molecular formula $C_{29}H_{48}O$; ESI-MS *m/z* 413.2 $[M+H]^+$; $^1$H-NMR ($CDCl_3$, 600 MHz); $^{13}$C-NMR ($CDCl_3$, 125 MHz).

*β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**5**): White powder; melting point: 274°C; molecular formula $C_{35}H_{60}O_6$; ESI-MS *m/z* 574.6 $[M-2H]^{2-}$, $^1$H-NMR (DMSO, 600 MHz); $^{13}$C-NMR (DMSO, 125 MHz).

## 3. Results and discussion

The structures of compounds 1-5 were described in Fig. 1.

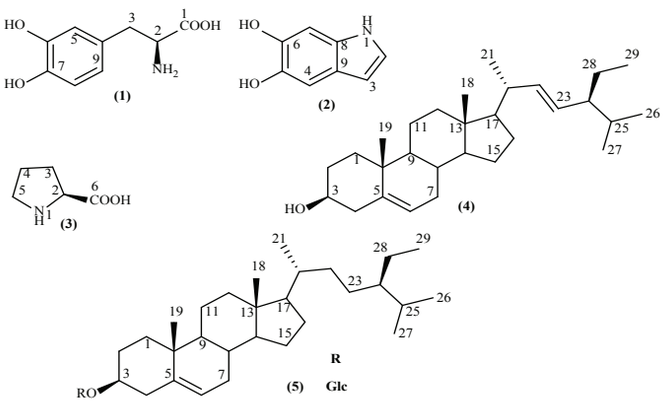


**Fig. 1. Structures of compounds 1-5.**

The white powder identified as compound **1** was separated from the watery crude extract. Based on the molecular ion-positive peak ESI-MS (*m/z* 197.8) $[M+H]^+$, the molecular formula of **1** was determined to be $C_9H_{11}NO_4$. The $^1$H-NMR spectrum showed the characteristic ABX system of the benzene ring at $\delta_H$ 6.85 (1H, d, *J*=8.4 Hz, H-8), 6.77 (1H, d, *J*=1.2 Hz, H-5), and 6.69 (1H, dd, *J*=8.4, 1.2 Hz, H-9). In addition, the $^1$H-NMR of **1** also showed a resonance signal of the methine proton at $\delta_H$ 4.07 (1H, m, H-2) and two doublets of doublet signals for methylene protons at $\delta_H$ 3.15

(1H, dd, *J*=5.4, 15.0 Hz, H-3a) and 2.84 (1H, dd, *J*=7.8, 15.0 Hz, H-3b). Combined analysis of $^{13}$C-NMR and HSQC of **1** revealed the presence of nine carbon signals, including a carboxylic acid at $\delta_C$ 172.6 (C-1), one methylene group at $\delta_C$ 35.2 (C-3), one aliphatic methine group at $\delta_C$ 54.9 (C-2), three unsaturated methine groups at $\delta_C$ 117.0 (C-5), 116.6 (C-8), and 121.9 (C-9) together with two quaternary carbons bonded to an oxygen atom at $\delta_C$ 144.3 (C-6), 143.6 (C-7), and one quaternary carbon at $\delta_C$ 126.9 (C-4). The HMBC of **1** showed correlations between H-8 and C-4, C-6, C-7 at $\delta_H$ 6.85 (1H, d, *J*=8.4 Hz, H-8)/$\delta_C$ 126.9 (C-4), 144.3 (C-6), 143.6 (C-7); H-5 and C-9, C-6, C-7 at $\delta_H$ 6.77 (1H, d, *J*=1.2 Hz, H-5)/$\delta_C$ 121.9 (C-9), 144.3 (C-6), 143.6 (C-7) along with H-9 and C-5, C-7, C-8 at $\delta_H$ 6.69 (1H, dd, *J*=8.4, 1.2 Hz, H-9)/$\delta_C$ 143.6 (C-7), 116.6 (C-8). Moreover, stronger correlations were observed between H-3a/H-3b and C-1, C-4, C-5, C-9 at $\delta_H$ 3.15 (1H, dd, *J*=5.4, 15.0 Hz, H-3a), 2.84 (1H, dd, *J*=7.8, 15.0 Hz, H-3b)/$\delta_C$ 172.6 (C-1), 126.9 (C-4), 117.0 (C-5), 121.9 (C-9) on HMBC of **1** indicating that the 2-amino-3-yl-propanoic moiety is linked to the benzene ring at C-4 position. The fairly large coupling constants of H-3a (*J*=5.4, 15.0 Hz) and H-3b (*J*=7.8, 15.0 Hz) are attributed to their equatorial-axial and axial-axial relationships with H-2, suggesting the axial orientation of H-2. Thus, compound **1** was elucidated as *L*-dopa or levodopa by comparison with data in the literature [7, 8].

The white powder identified as compound **2** was isolated from the crude ethyl acetate extract. Its molecular formula was deduced as $C_8H_7NO_2$ based on the spectroscopic data of HRESI-MS with the molecular protonated molecular ion peak at (*m/z* 150.0251) $[M+H]^+$ in the positive HRESI-MS spectrum. Two singlet signals at $\delta_H$ 6.93 (1H, s, H-4) and 6.83 (1H, s, H-7), which are characteristic signals of an aromatic ring system, were found in the $^1$H-NMR of **2**. In addition, the $^1$H-NMR of **2** also exhibited two signals, each being a doublet at $\delta_H$ 6.99 (1H, d, *J*=3.0 Hz, H-2), 6.21 (1H, d, *J*=3.0 Hz, H-3). Combined analysis of $^{13}$C-NMR and HSQC of **2** revealed eight carbon signals comprising four methines (4×CH) at $\delta_C$ 123.7 (C-2), 101.5 (C-3), 105.6 (C-4), 97.9 (C-7) and four quaternary carbons (4×Cq) at $\delta_C$ 143.6 (C-5), 141.3 (C-6), 132.3 (C-8), 122.5 (C-9). This evidence suggests that the structure of **2** is created by a benzene and a pyrrole ring, or an indole skeleton. The linkage positions of the benzene ring with the pyrrole ring at C-8 and C-9 can be inferred by correlations on the HMBC of **2**. The correlations between H-2 and C-3, C-8, C-9 at $\delta_H$ 6.99 (1H, d, *J*=3.0 Hz, H-2)/$\delta_C$ 101.5 (C-3), 132.3 (C-8), 122.5 (C-9); H-3 and C-2, C-8 at $\delta_H$ 6.21 (1H, d, *J*=3.0 Hz, H-3)/$\delta_C$ 123.7 (C-2); 132.3 (C-8), which confirmed the connecting position of the pyrrole ring with the benzene ring at C-8 and C-9 or an indole skeleton of **2**. Moreover, the correlation between of H-4 and C-3, C-5; C-6; C-8 at $\delta_H$ 6.93 (1H, s, H-4)/$\delta_C$ 101.5 (C-3), 143.6 (C-5), 141.3 (C-6), 132.3 (C-8); H-7 and C-5; C-6; C-9 at $\delta_H$ 6.83 (1H, s, H-7)/$\delta_C$143.6 (C-5), 141.3 (C-6), 122.5 (C-9) were also recognised by the HMBC of **2**. Spectroscopic data analysis (1D, 2D-NMR, HRESI-MS) and comparison with published literature [9] identified compound **2** as 5,6-dihydroxyindole.

Compound **3** was isolated from the aqueous crude extract as a white powder. The positive ESI-MS of **3** showed a pseudo-molecular ion peak at *m/z* 115.9 $[M+H]^+$, suggesting a molecular formula of $C_5H_9NO_2$. A doublet of doublets of the methine proton at $\delta_H$ 4.00 (1H, dd, *J*=6.0, 8.4 Hz, H-2) and three methylene protons at $\delta_H$ 3.40 (1H, m, H-5a), 3.27 (1H, m, H-5b), 2.30 (1H, m, H-3a), 2.14 (1H, m, H-3b), 2.00 (2H, m, H-4) were revealed on the $^1$H-NMR of **3**. Combining $^{13}$C-NMR and HSQC analyses of **3** showed five signals, encompassing three methylenes, one methine, and one carboxylic acid. This data suggested that compound **3** is the amino acid *L*-proline. The HMBC of **3** revealed the correlations of H-2 with C-3, C-4, C-6 at $\delta_H$ 4.00 (1H, dd, *J*=6.0, 8.4 Hz, H-2)/$\delta_C$ 30.4 (C-3), 25.1 (C-4), 174.0 (C-6) and of H-3a (H-3b) with C-6 at $\delta_H$ 2.30 (1H, m, H-3a), 2.14 (1H, m, H-3b)/$\delta_C$ 174.0 (C-6) which illustrated that carboxyl group (-COOH) attached with pyrrolidine ring at C-2 position. Moreover, the relatively large coupling constant of H-2 (*J*=6.0, 8.4 Hz) confirmed its axial-equatorial and axial-axial relationships with H-3a and H3b, indicating the axial orientation of H-2. Based on its $^1$H-, $^{13}$C-NMR, and 2D-NMR data, compound **3** was determined to be *L*-proline [10, 11].

Compound **4** was separated from the ethyl acetate crude extract as a white powder. The $^1$H-NMR of **4** showed a triplet at $\delta_H$ 5.35 (1H, t, *J*=3.0 Hz, H-6); an oxygenated methine proton at $\delta_H$ 3.52 (1H, m, H-3); and two olefinic protons at $\delta_H$ 5.16 (1H, dd, *J*=8.4, 15.0 Hz, H-22), and 5.02 (1H, dd, *J*=9.0, 15.0 Hz, H-23) together with six methyl signals at $\delta_H$ 1.02 (3H, d, *J*=6.6 Hz, H-21), 1.01 (3H, s, H-19), 0.85 (3H, d, *J*=6.0 Hz, H-27), 0.80 (3H, t, *J*=7.2 Hz, H-29), 0.79 (3H, d, *J*=6.5 Hz, H-26), and 0.69 (3H, s, H-18). The $^{13}$C-NMR and DEPT spectra of **4** displayed a total of 29 carbons, comprising six methyls (6x$CH_3$), nine methylenes (9x$CH_2$),

eleven methines (11xCH), and three quaternary carbons (3xCq). Compound **4** has been confirmed as stigmasterol through its spectrometric analysis and comparison with previously published data [12].

Compound **5** was isolated from the ethyl acetate crude extract as a white powder. The $^{1}$H and $^{13}$C-NMR of **5** closely resembled those of compound **4**, except for the appearance of one anomeric proton at $\delta_H$ 4.22 (1H, d, *J*=7.8 Hz; H-1′)/$\delta_C$ 100.7 (C-1’) and disappearance of two olefinic protons at $\delta_H$ 5.02 and 5.16. This suggested that **5** contains a sterol with a β-sitosterol aglycone moiety linked to a β-glucopyranose unit. Based on its negative ESI-MS, NMR data, and comparison with reported data, **5** was elucidated as *β*-sitosterol-3-*O*-*β*-D-glucopyranoside [13].

## 4. Conclusions

Five compounds were isolated from the seed of *Mucuna pruriens*, comprising three nitrogenous compounds: *L*-dopa (**1**), 5,6-dihydroxyindole (**2**), *L*-proline (**3**), along with two known sterols, stigmasterol (**4**) and β-sitosterol-3-*O*-β-D-glucopyranoside (**5**). The structures of compounds **1-5** were elucidated by ESI-MS, HRESI-MS, and NMR. This is the first report on the isolation of compounds **2** and **3** from the seeds of this species.

## CRediT author statement

Nguyen Van Tai: Supervision, Funding acquisition, Data curation, Principal investigator; Phan Thi Trang: Investigation, Formal analysis; Nguyen Thi Thu Trang: Investigation, Formal analysis; Nguyen Thi May: Conceptualisation, Methodology, Formal analysis; Le Thi Loan: Conceptualisation, Formal analysis; Nguyen Thi Hong Anh: Methodology, Formal analysis; Nguyen Minh Khoi: Investigation, Formal analysis, Resources; Dang Viet Hau: Data curation, Principal investigator, Writing - Original draft preparation.

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## COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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