

Original Article

Isolation of a sterol from root of *Mucuna pruriens*

Pramod Kumar^{a,*}, Vijay Juyal^b, Showkat R. Mir^c

^aDepartment of Pharmaceutical Sciences, H.N.B. Garhwal University, Srinagar, Garhwal, Uttarakhand, India.

^bDepartment of Pharmaceutical Sciences, Kumaun University Bhimtal, Nainital, Uttarakhand, India.

^cDepartment of Pharmacognosy & Phytochemistry, Jamia Hamdard University, New Delhi, India.

*Corresponding Author. Tel.: +91 9456532849, E-mail address: pramodhnbgu@gmail

ARTICLE INFO

Received 15 Nov 2014

Revised 28 Nov 2014

Accepted 15 Dec 2014

Keywords:

- FTIR
- NMR
- Column Chromatography
- Soxhlet apparatus

ABSTRACT

Mucuna pruriens belongs to the family Fabaceae significantly used in Indian traditional system for their aphrodisiac, antidiabetic, antioxidant, antihyperlipidemic, anticancer and various other human ailments. The biological activity of mucuna roots has been reported in various research papers. The present paper reveals the isolation of a sterol. On the basis of spectral data and chemical reactions, the structure of compound was elucidated as 3 β -benzoxy-stigmast 5-ene.

1. INTRODUCTION

Mucuna pruriens belongs to the family Fabaceae commonly known as Kiwanch or konch in Hindi, cowitch in English and kapikacho in Sanskrit is the most popular drug in Ayurvedic and Unani system of medicine in India and Bangladesh. The plant is being cultivated in Bangladesh, India, Sri Lanka, South East Asia and Malaysia [1-7]. All parts of *M. pruriens* are generally used to treat impotence [8] diabetes mellitus [9] and cancer [10]. The seeds have multi-diversified functions like free radical mediated diseases management, rheumatoid arthritis, diabetes, atherosclerosis, nervous disorders and parkinsonism [11]. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins, phenolic compounds and sterol [12]. The chemical constituents may be used for the various purposes such as activity against pathogenic bacteria [13].

2. MATERIAL AND METHODS

1. All the chemicals and reagents were obtained from s.d. fine chemicals of analytical grade.
2. Sodium sulfate was used as drying agent for various solvents used to run the column.

3. All the weighing was done on a single pan Mettler balance.
4. Melting points were determined on Perfit melting apparatus.
5. Infra red spectra were recorded on Shimadzu FTIR spectrophotometer using KBr pellets; ν_{\max} values are given in cm^{-1} .
6. ¹H NMR spectra were screened on advance DRY 400, Bruker spectropin 400 MHz instrument using CDCl₃ as solvent and TMS as an internal standard. Chemical shift values are given in δ (ppm) scale and coupling constants (J) in Hz. Notations used throughout as s= singlet, d= doublet, dd= double doublet, t= triplet, m= multiplet and brs= unresolved broad singlet.
7. ¹³C NMR spectra were recorded on advance DRY 400, Bruker spectropin 100 MHz in 5 mm spinning tubes at 27°C.
8. Mass spectra's were scanned by effecting electron Impact ionization at 70 eV on a JEOL-JMS-DX303 instrument equipped with direct inlet probe system. The m/z values of the more intense peaks were mentioned and the figures in brackets attached to each m/z value indicate relative intensities with respect to the base peak.

Collection and authentication of plant material.

The roots of *Mucuna pruriens* were collected from Chouras campus H.N.B. Garhwal University Sringer Garhwal in the October 2010, and were identified by Dr. R. M. Painuli, Department of Botany, and H.N.B. Garhwal University Sringer Garhwal. A specimen is for further reference has been retained in the department.

Extraction of plant material

Roots of *M. pruriens* were shade dried for a week. Roots were coarsely powdered and methanolic extract was prepared using soxhlet apparatus. Extract was concentrated under reduced pressure to yield dark brown viscous syrupy mass and mixed slowly with silica gel until it becomes free flowing. Lower end of columns was plugged with absorbent cotton over which a piece of cotton was placed. The column was then half filled with petroleum ether; silica gel was added in small portions and allowed to settle down gently until the necessary length of column was attained. All the air bubbles were allowed to escape by running the column continuously with solvent. The silica gel slurry of the extracts were packed in the column and then eluted successively in order of increasing polarity with different solvents.

3. RESULT AND DISCUSSION

Elution of column with Pet ether- Chloroform (1:1) eluants resulted in isolation of a waxy compound. M.P. 250-260 and R_f Value 0.5 (solvent system Chloroform : Methanol

Table 1. ^{13}C -NMR spectra of 3 β -benzoxy-stigmast-5-ene

C	δc	C	δc
1	36.9	21	13.6
2	28.4	22	31.4
3	72.9	23	34.5
4	39.8	24	37.9
5	139.1	25	28.1
6	118.9	26	19.2
7	25.5	27	22.9
8	35.0	28	23.5
9	55.1	29	16.5
10	29.1	30	-
11	22.2	1'	172.3
12	24.2	2'	143.5
13	40.5	3'	128.0
14	39.8	4'	130.3
15	33.4	5'	126.8
16	36.6	6'	127.9
17	52.3	7'	135.8
18	15.7	38	
19	19.5		
20	30.9		

5:3v/v) FTIR (KBr) V_{max} 2954, 2873, 1705, 1645, 1602 1515, 1450, 1382, 1253, 1124, 1045 cm^{-1} . ^1H NMR (DMSO- d_6) δ 8.12 (2H, dd, $J=7.1, 1.8\text{Hz}$, H-4',6'), 7.56 (1H, dd, $J=7.2, 1.8\text{Hz}$, H-5'), 6.96 (2H, t, $J=7.1\text{ Hz}$, H-3',7'), 5.41 (1H, brs, H-6), 4.51 (1H, brs, H-3), 1.22 (3H, d, $J= 9.1\text{Hz}$, H3-21), 1.03 (6H, d, $J=6.3\text{Hz}$, H3-26,27), 0.99 (3H, t, $J=7.1\text{Hz}$, H3-29), 0.84 (3H, s, H3-19), 0.63 (3H, s, H3-18)+Ve ES-MS M/z (Rel. int. %) 520(M $^+$) $\text{C}_{36}\text{H}_{54}\text{O}_2$ (2.1), 506 (6.2) 414 (100), 396 (45), 302 (13) 274 (27) 259 (41)

Compound was obtained as waxy mass from Pet ether-chloroform (3:1) eluants. It gave positive result in salkowski test for sterols. Its FTIR spectrum (Figure 1) exhibited absorption bands characteristic for ester (1705 cm^{-1}) and aromatic moiety (1602, 1515 cm^{-1}). On the basis of ^{13}C NMR and mass spectra, its molecular weight was found to be 518, consistent with the molecular formula $\text{C}_{36}\text{H}_{54}\text{O}_2$ of a benzyl ester of sitosterol. Its ES-MS spectra displayed peaks at m/z 414 and other peaks characteristic of sitosterol. The ^1H NMR spectrum of compound exhibited two downfield double –doubles at δ 8.12 ($J=7.0, 1.8\text{Hz}$) δ 7.56 ($J= 7.1, 1.8\text{Hz}$), integrating for two and one proton, were correspondingly ascribed to H-4', H-6' and H-5' ortho-meta coupled aromatic protons. A two proton triplet at δ 6.96 ($J=7.1\text{ Hz}$) was associated with H-3' and H-7' aromatic protons. A single proton broad signal at δ 5.41 was assigned to H-3 α carbinol proton. Two doublets at δ 1.22 (3H, $J=9.1\text{Hz}$) and 1.03 (6H, $J=6.3\text{ Hz}$) were correspondingly attributed to H₃-30, H₃-26 and H₃-27 methyl protons. A three –proton triplet at δ 0.99 ($J=7.1\text{ Hz}$) and two three-proton singlet at δ 0.84 and 0.63 were ascribed to H₃-29, H₃-19 and H₃-18 methyl proton, respectively. The ^{13}C NMR spectrum of compound exhibited thirty six signals. The important signals appeared for ester carbon at δ 172.3 (C-1'); vinylic carbon at δ 139.1 (C-5), 118.9 (C-6); aromatic carbon from δ 143.5 to 126.8, and carbinol carbon at δ 72.9. On the basis of above spectra data and chemical reactions, the structure of compound was elucidated as 3 β -benzoxy-stigmast 5-ene (Figure 5).

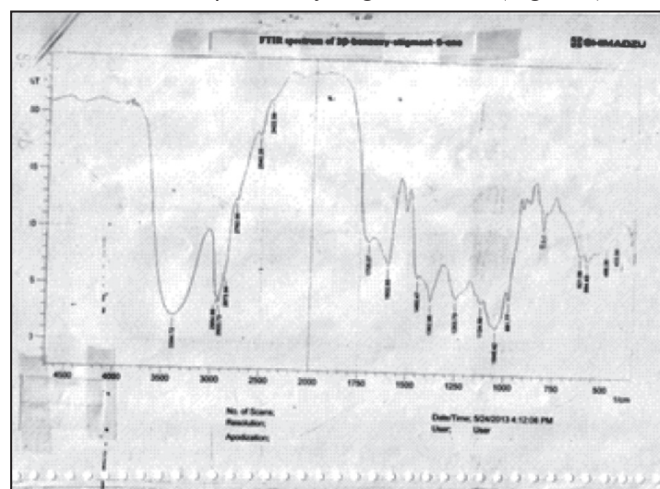


Fig. 1. FTIR data of 3- β - benzoxy-stigmast-5-ene

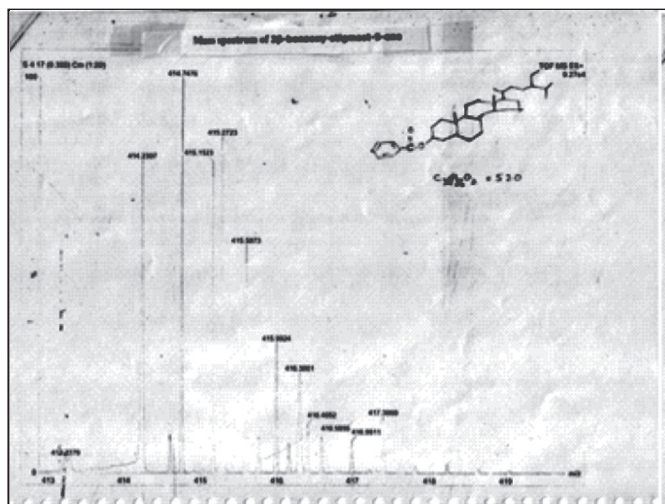


Fig. 2. Mass spectrum of 3-β- benzyoxy-stigmast-5-ene

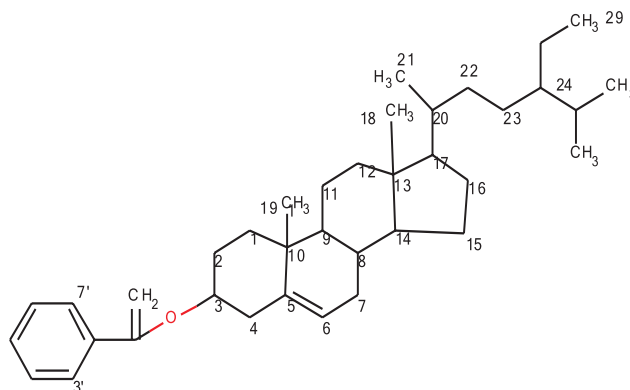


Fig. 5. 3 β- Benzyoxy-stigmast-5-ene

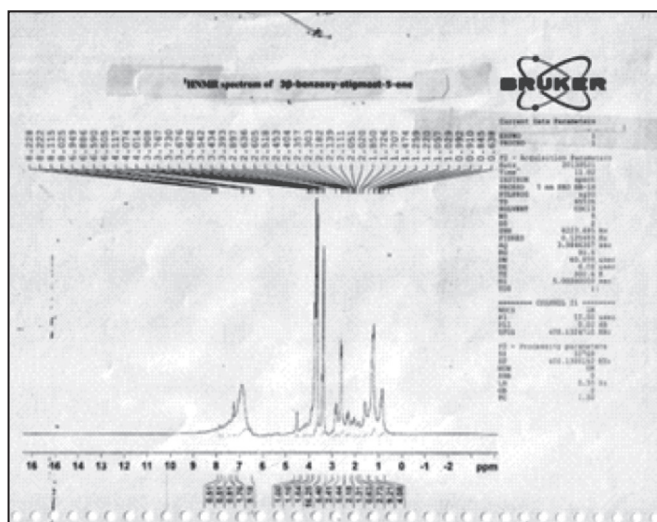


Fig. 3. ¹H-NMR spectrum of 3-β- benzyoxy-stigmast-5-ene

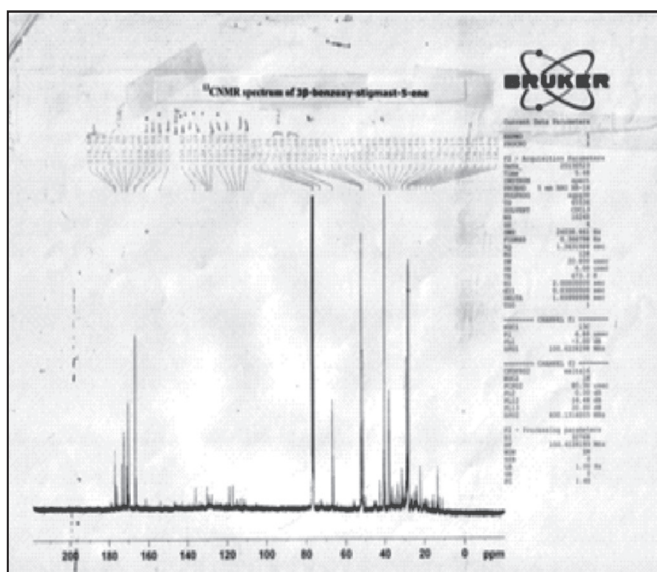


Fig. 4. ¹³C NMR spectrum of 3-β- benzyoxy-stigmast-5-ene

4. CONCLUSION

Mucuna roots have wide variety of biological activity. So there is possibility to isolate various phytoconstituents which might be responsible for biological activity.

Acknowledgements

I sincerely thankful to Department of Pharmaceutical Sciences, H.N.B. Garhwal University, Srinagar- Garhwal, Uttarakhand to provide necessary facility for isolation of compound. I am really thankful to Dr. Showkat R. Mir, Faculty of Pharmacognosy & Phytochemistry, Jamia Hamdard New Delhi for the interpretation of the spectral data.

REFERENCES

- [1] Maiti, R.; Jana, D.; Das, U.K.; Ghosh, D. Antidiabetic effect of aqueous extract of seed of *tamarindus indica* in streptozotocin induced diabetic rats. *J Ethnopharmacol* 2004, 92, 85-91.
- [2] Wadkar, K.A.; Magdum, C.S.; Patil, S.S.; Naikwade, N.S. Antidiabetic potential and Indian medicinal plants. *J Herbal Med and Toxicol* 2008, 2, 45-50.
- [3] Welihinda, J.; Arvidson, G.; Gylfe, E.; Hellman, B.; Karlsson E.Ada. *Biol MetLGer* 1982, 41, 1229.
- [4] Hongxiang H.; George T.; Vay Liang W Go. VLW. Hypoglycemic herbs and their action mechanisms. *Chin Med* 2000 4, 11-14.
- [5] Liu, I.M.; Tzeng, T.F.; Liou, S.S.; Lan, T.W. Improvement of insulin sensitivity in obese Zucker rats by myricetin extracted from *Abelmoschus moschatus*. *Planta Med* 2007, 73, 1054-1060.
- [6] Wadood, A.; Wadood, N.; Shah, S. A. Effects of *Acacia arabica* and *Caralluma edulis* on blood glucose levels on normal and alloxan diabetic rabbits. *J Pakistan Med* 1989, 39, 208-212.

- [7] Akhtar, M.S.; Iqbal, J. Evaluation of the hypoglycaemic effect of *Achyranthes aspera* in normal and alloxan- diabetic rabbits. *J Ethnopharmacol* 1991, 31, 49-57.
- [8] Ruffa, M.J.; Ferraro, G.; Wagner, M.L.; Calcagno, M.L.; Campos, R.H.; Cavallaro, L. Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. *J Ethnopharmacol* 2002, 79, 335-339.
- [9] Kadarian, C.; Broussalis, A.M.; Miño, J.; Lopez, P.; Gorzalczany, S.; Ferraro, G.; Acevedo C. Hepatoprotective activity of *Achyrocline satureioides* (Lam) D. C. *Pharmacol Res* 2002, 45, 57-61.
- [10] Andrade-Cetto, A.; Wiedenfeld, H. Hypoglycemic effect of *Acosmium panamense* bark on streptozotocin diabetic rats. *J Ethnopharmacol* 2004, 90, 217-220.
- [11] Ponnachan, P.T.; Paulose, C.S.; Panikkar, K.R. Effect of leaf extract of *Aeglemannelose* in diabetic rats. *Indian J Exp Biol* 1993, 31, 345-347.
- [12] Gray, A.M.; Flatt, P.R. Actions of the traditional anti-diabetic plant, *Agrimony eupatoria* (agrimony): effects on hyperglycaemia, cellular glucose metabolism and insulin secretion. *Br J Nutr* 1998, 80, 109-114.
- [13] El Hilaly, J.; Lyoussi, B. Hypoglycaemic effect of the lyophilised aqueous extract of *Ajuga ivain* normal and streptozotocin diabetic rats. *J Ethnopharmacol* 2002, 80, 109-113.