



INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Allantoin from the leaves of *Pisonia grandis* R.Br.

Shubashini Krishnan Sripathi*, Poongothai Gopal and Pottail Lalitha

Department of Chemistry, Avinashilingam Deemed University for Women, Coimbatore, TN - (India)

Abstract

Phytochemical investigation of the leaves of the *Pisonia grandis* afforded three compounds (1-3). Compound 1 was identified as the insulin mimetic molecule pinitol and its isolation was reported in our earlier paper¹. The present paper deals with the isolation of compounds 2 and 3, whose structures were established as that of allantoin and its hydrogen bonded isomeric crystalline form, based on spectroscopic data and it is the first report of isolation of allantoin and its hydrogen bonded isomer from *Pisonia* genus and from this species.

Key-Words: *Pisonia grandis*; *Nyctaginaceae*; Allantoin; NMR.

Introduction

The species *Pisonia grandis* R.Br (*Nyctaginaceae*) is widely distributed throughout India and is a widespread evergreen commonly grown lettuce tree and is especially adapted to sea coasts and grows well in gardens in Chennai and other places near the sea, on both east and west coasts². Leaves, stem and root of this species are extensively used by the tribals in the preparation of several folk medicines. It has been extensively used in Indian traditional medicine as an antidiabetic, anti-inflammatory agent, and used in the treatment of algesia, ulcer, dysentery and snake bite³⁻⁸. The plant has been studied by different workers with special reference to its pharmacological activity but no isolation of phytochemicals has been reported⁹. Hence phytochemical investigation of the leaves of *Pisonia grandis* was undertaken.

Material and Methods

The plant material (leaves) was collected during January-March 2009 from the local areas of Coimbatore, Tamilnadu, India. The identity of plant material was confirmed by the taxonomist Dr. C.Kunhikannan, Scientist D, Biodiversity Division, Institute of Forest Genetics & Tree Breeding, Coimbatore. The leaves were dried in shade and cut into small pieces and then used for phytochemical study. Air dried pieces of leaves of *Pisonia grandis* were extracted with 100% ethanol for 6 hour at reflux temperature. The extract was filtered; the filtrate was evaporated to dryness.

The residue obtained from the ethanol extract (about 35 g) was dissolved in minimum quantity of methanol and made into slurry with minimum amount of silica gel and subjected to chromatographic separation over a column of silica gel (400 g) built in chloroform. The column was eluted with i) chloroform, ii) chloroform and methanol mixtures with increasing amounts of methanol. Eluates of 200 ml were collected and the solvent was distilled off on a water-bath. The homogeneity of the fractions was examined by TLC on silica gel plates by suitable mobile system. Similar fractions were combined and crystallized. The chromatographic analysis led to the isolation of three compounds 1-3. The isolated compounds were characterized by preliminary tests and spectral studies. The characterization of compound 1 has been dealt with in our earlier paper¹.

Results and Conclusion

Compound 2 was isolated from the chloroform: methanol (85:15) eluate of the silica gel column of the ethanol extract. Its melting point is 218-224°C. The compound gave a bright blue solution on Lassaigne's test for nitrogen characteristic of urea-like compounds. It was negative to other color tests meant for the common class of compounds. IR spectrum of the compound gave a characteristic absorption due to carbonyl group (1780 cm⁻¹), and it indicated the presence of -NH grouping also (3438 cm⁻¹).

The ¹H NMR spectrum of 2 expressed fewer signals, thus indicating a simple structure for the compound. ¹H NMR spectrum gave very prominent peaks at δ 5.24 - 5.25 (doublet, 1H) δ 5.83 (singlet, 2H), δ 6.96- 6.98 (doublet, 1H), δ 8.06 (singlet, 1H) and δ 10.54 (broad singlet, 1H). The D₂O wash ¹H NMR spectrum

*** Corresponding Author:**

E-mail: adusks@gmail.com
Mob.: 098422 67798

of 2 revealed that all the signals except that at δ 5.24 - 5.25 reduced in intensity while this signal showed a threefold increase in intensity (from intensity 1.02 units shown in the ^1H NMR spectrum of 2 to intensity 3.72 units shown in its D_2O wash ^1H NMR spectrum). Also the signal at δ 5.83 corresponds to 2 protons and its intensity is also considerably reduced in the deuterium exchanged ^1H NMR spectra.

The broadband ^{13}C NMR spectrum of 2 showed 4 distinct signals at δ 62.90, δ 157.28, δ 157.96 and δ 171.04. The DEPT 90 NMR spectrum of 2 revealed the presence of only one signal at δ 62.90 indicating one methine group only. The ^1H - ^1H COSY spectrum of 2 helped in finding the useful correlation between the mutually coupling protons. Off diagonal elements were present at δ 5.24 and δ 6.96, at δ 5.24 and δ 8.0 which indicate that the protons at these positions are mutually coupling partners. However the signal contour at δ 8.06 was small. Probably a weak coupling interaction may be present between the proton at δ 5.24 and the proton at δ 8.06.

The ^1H - ^{13}C HETCOR spectrum (HSQC) of 2 showed only one contour correlating the proton signal at δ 5.24 to the carbon signal at δ 62.90. Hence the other proton signals are due to proton attached to hetero atoms. There are four such signals in the ^1H NMR spectrum. A thorough analysis of all the spectra and comparison of spectral data with that in literature revealed that compound 2 is the heterocyclic compound Allantoin¹⁰. Table 1 shows the comparison of ^1H NMR and ^{13}C NMR data made with literature data¹¹ and it highlights the agreement. This is the first report of isolation of allantoin from *Pisonia* genus and from this species also.

Compound 3 was isolated from the chloroform:methanol (94:6, 92:8 and 90:10) eluate of the silica gel column of the ethanol extract (Melting point $> 250^\circ\text{C}$). The ^1H NMR spectrum of 3 expressed signals at δ 5.25-5.27 (doublet, 1H), δ 5.85 (singlet, 2H), δ 7.04-7.05 (doublet, 1H), δ 8.07 (singlet, 1H), and δ 10.58 (singlet, 1H). The ^1H NMR spectrum of 3 resembled that of 1 in most aspects. The broad band ^{13}C NMR spectrum of 3 showed two distinct signals at δ 62.90 and δ 157.37. Two weak signals were observed at δ 174.61 and δ 158.22. Although the observed ^{13}C signals for 3 appear around the same chemical shift values as that of 2, one weaker signal was shown at δ 66.35. The DEPT 90 NMR spectrum of 3 revealed only one signal at δ 62.89 indicating the presence of only one methine group in 3. The DEPT 135 NMR spectrum also showed only one signal at δ 62.89. A striking difference between the ^1H - ^1H COSY spectrum of 3 and 2 was noticed. The ^1H - ^1H COSY spectrum of 3

revealed an additional weak coupling interaction between the protons at δ 8.07 and that at δ 10.58. This was not seen in the COSY spectrum of 2. It has been reported that the most interesting feature of the structure of allantoin is the hydrogen bonding which involves three carbonyl-oxygens, three imido and two amino hydrogen atoms and links the molecule into an intricate three dimensional network¹². The spectral analysis of 3 revealed that two of its carbonyl signals were masked in intensity and the compound did not give a bright blue coloured solution in Lassaigne's test characteristic of urea-like compounds. It gave only pale green colouration in this test. Hence the above significant observations suggested that compound 3 may be a hydrogen bonded isomeric crystalline form of 2 whose structure has been proposed as allantoin (Fig.1.)

The compounds 2 and 3 have been isolated for the first time from *Pisonia* genus and from the medicinal plant *Pisonia grandis* and are reported to have pharmacological significance. The antifungal activity of *Pisonia grandis* has been studied in the same laboratory. The ethanol extract of the plant showed significant antifungal activity¹³ and the present isolation of allantoin (Patent Pending No.3606/CHE/201) and its hydrogen bonded derivative from this plant justifies its antifungal potential. Hence the present investigation finds immense phytochemical significance.

Acknowledgements

The authors thank the authorities of Avinashilingam University for Women, Coimbatore, India for providing necessary facilities. Authors are also grateful to The Sophisticated Analytical Instruments Facility, Indian Institute of Technology Madras, India for the spectral data.

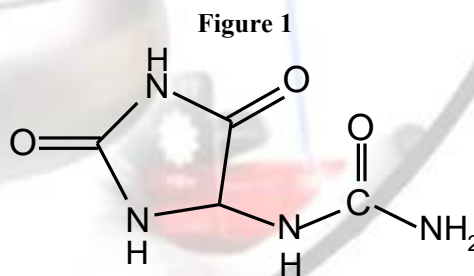


Table 1: Comparison of ¹H NMR and ¹³C NMR data of 2 with the literature values

Proton position	Chemical shift in δ	Literature values	Carbon position	Chemical shift in δ	Literature values
H-1	10.54 (br.s) 1H	10.50 (s) 1H	C2	157.28	157.1
H-3	6.96-9.68 (d) 1H	6.9 (d) 1H	C4	62.90	62.7
H-4	8.06 (s) 1H	8.1 (s) 1H	C5	171.04	174.0
H-6	5.24-5.25 (d) 1H	5.3 (d) 1H	C7	157.96	157.6
H-8	5.83 (s) 2H	5.83 (s) 2H			

References

- Shubashini K. Sripathi, Poongothai G and Lalitha P. (2011). Anti diabetic agent Pinitol from the leaves of *Pisonia grandis* (R.Br.), *Journal of Natural Remedies*, **11**(1): 39-43
- Anonymous (1969) *The Wealth of India – An Encyclopedia of India's Raw material Resources*. Edn 1, VIII (Ph-Re)
- Sunil C., Latha P.G., Suja S.R., Shine V.J, Shyamal S., Anuja G.I., Sini S., Rajasekharan S., Ajastian P., Ignacimuthu S. and Kaliselvan V. (2009). Effect of ethanolic extract of *pisonia alba* Span. Leaves on blood glucose levels and histological changes in tissues of alloxan-induced diabetic rats, *International Journal of Applied Research in Natural products*, **2**(2):4-11.
- Radha R., Arokiyaraj S., Agastian P., Balaraju K., Mohan Kumar R. and Bula P. (2008). Phytochemical analysis and anti-inflammatory activity of *Pisonia grandis* R.Br., *Journal of Biomedical and Pharmacology*, **1**:1.
- Prabu D., Nappinnai M., Ponnudurai K. and Prabhu K. (2008). Evaluation of Wound-Healing Potential of *Pisonia grandis* R.Br: A Preclinical Study in Wistar Rats, *The International Journal of Lower Extremity Wounds*, **7**(1): 21-27
- Anbalagan N., Rajinikanth K.N., Kishore Gnanasam S., Thomas Leonard J., Balakrishna K., Ramachandran S. and Sridhar S.K. (2002) Analgesic, anti-inflammatory and Diuretic Activities of *Pisonia grandis*. *Journal of Natural Product Sciences*, **8**(3):97-99.
- Will McClatchey, (1996). *Journal of Ethnopharmacology*, **50**(3):147-156.
- <http://www.Dukesphytochemicaldatabase>
- Buenz E.J., Johnson H.E., Beekman E.M., Motley T.J. and Bauera B.A. (2005). Bioprospecting Rumphius's *Ambonese Herbal*: Volume I. *Journal of Ethnopharmacology*, **96**: 57-70.
- Asia NR, Fatma UA, Mayadesh shaedah, Mutasemo OT. (2004) Investigation of the active constituents of *portulaca oleraceae* L. (*Portulacaceae*) growing in jordan. *Pakistan Journal of Pharmaceutical Sciences*, **17**(1):37-45.
- Asia N. Rashed., Fatma U. Afifi., Mayades Shaedah and Mutasem O. Taha (2004). Investigation of the active constituents of *portulaca oleraceae* L. (*Portulacaceae*) growing in Jordan, *Pakistan Journal of Pharmaceutical Sciences*, **17**(1):37-45.
- Mootz, D. (1965). The crystal structure of DL-Allantoin, *Acta Cryst*, **19**:726-734.
- Shubashini K.Sripathi and Poongothai G. (2010). Bio-assay guided fractionation and anti-fungal activity studies on *Pisonia grandis* (R.Br.), *International Journal of Current Research*, **10**: 35-37

