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

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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 paper[1]. 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 coasts[2]. 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 bite[3-8]. The plant has been studied by different workers with special reference to its pharmacological activity but no isolation of phytochemicals has been reported[9]. 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 Breading, 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.

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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 paper[1].

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 ^{1}HNMR spectrum of 2 expressed fewer signals, thus indicating a simple structure for the compound. ^{1}H 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 D_2O wash ^{1}H NMR spectrum

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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 ^{1}H NMR spectrum of 2 to intensity 3.72 units shown in its D_2O wash ^{1}H NMR spectrum). Also the signal at δ 5.83 corresponds to 2 protons and its intensity is also considerably reduced in the deuterium exchanged ^{1}H 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 ^{1}H- ^{1}H 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 ^{1}H- ^{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 ^{1}H 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 Allantoin [10]. Table 1 shows the comparison of ^{1}H NMR and ^{13}C NMR data made with literature data[11] 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 °C). The ^{1}H 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 ^{1}H 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 ^{1}H- ^{1}H COSY spectrum of 3 and 2 was noticed. The ^{1}H- ^{1}H 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 network[12]. 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 allantion (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 activity [13] 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.

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Figure 1

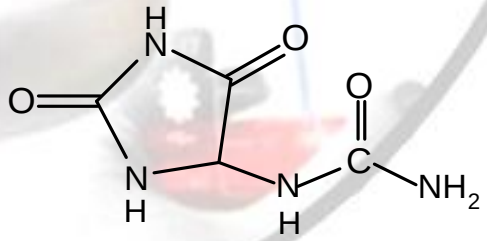

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Table 1: Comparison of ^{1}H NMR and ^{13}C NMR data of 2 with the literature values

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			

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