Journal of Saudi Chemical Society (2016) **20**, 81–87

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Isolation and quantification of pinitol in *Argyrolobium roseum* plant, by ^{1}H-NMR

Neha Sharma [a], Mahendra K. Verma [a,*], Devinder K. Gupta [b], Naresh K. Satti [b], Ravi K. Khajuria [a]

[a] *Analytical Chemistry Division (Instrumentation), CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-Tawi 180001, India*
[b] *Natural Product Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-Tawi 180001, India*

Received 5 August 2013; revised 2 July 2014; accepted 9 July 2014
Available online 17 July 2014

KEYWORDS

Argyrolobium roseum;
q-NMR;
Relaxation;
Pinitol;
Pyrazinamide

Abstract Chemical investigations on ethanolic extract of *Argyrolobium roseum* led to the isolation of Pinitol as the major constituent of the plant. Pinitol is chemically known as 3-O-methyl-D-Chiro-inositol and has been found to possess anti-diabetic activity. It helps in the regeneration of beta cells, present in the areas of the pancreas called as islets – of Langerhans. These cells make and release insulin, a hormone which controls the level of glucose in the blood. Pinitol was isolated from the ethanolic extract of the plant and a sensitive & reliable method, based on Proton Nuclear Magnetic Resonance (PNMR), was developed and used as an analytical tool for quantification and identification of this relatively UV insensitive compound in the alcoholic extract of the plant. The method involves the use of pyrazinamide (an anti-tuberculosis drug), as a reference. Validation of the method was carried out by preparing a known concentration of an artificial mixture of pinitol and pyrazinamide. The recovery of pinitol in the mixture was in the range of 98.5–101.3%. Pinitol in pure form was isolated from the ethanolic extract of *A. roseum* by repeated column chromatography over silica gel followed by crystallization in methanol. Pinitol isolated from the plant was identified on the basis of ^{1}H-NMR, ^{13}C-NMR, DEPT (45°, 90° and 135°) experiments and mass spectral data. The method was successfully applied for the quantitation of pinitol in various extracts of the said plant.

1. Introduction

Pinitol (Fig. 1) or 3-O-methyl D-Chiro-inositol, has been reported to possess antidiabetic and hypoglycaemic activities [1,2]. It has been claimed that the compound possesses insulin like effects. The studies have revealed that D-pinitol can act like insulin to improve glycaemic control in hypoinsulinaemic STZ-diabetic mice [3]. Inositol phosphoglycans are potentially important post-receptor mediators of insulin action. Pinitol

* Corresponding author. Address: CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu (J&K) 180001, India. Tel.: +91 191 2569010x229.
E-mail addresses: mkvermadr@yahoo.com, mkverma@iiim.ac.in (M.K. Verma).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2014.07.002

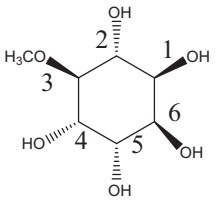

Figure 1 Pinitol.

has been found to mediate certain actions of insulin [4]. Lot of work has been reported in the literature on pinitol and its analogues [5–8]. The compound has shown significant hypoglycaemic and antidiabetic activities in normal and alloxan – induced diabetic albino mice and is free from acute toxicity. Screening of *Argyrolobium roseum* for its chemical constituents led to the isolation of pinitol (Fig. 1) as the major constituent of the plant. This is a virgin plant, and no reference regarding its chemical constituents was found in the literature. As pinitol showed very poor response to the HPLC-UV (DAD) method, we decided to quantify this compound in the ethanolic extract of the plant by Proton Magnetic Resonance Spectroscopy while using pyrazinamide (Fig. 2) as a reference compound.

Proton Magnetic Resonance (PNMR) spectroscopy is becoming of great importance [9–16] in the determination and quantitative analysis of natural products, food products and drug metabolites. The plant extract is a biogenetic cocktail and some of the components in this biogenetic cocktail show poor response to commonly used Diode Array, UV/VIS and Fluorescent detectors. There is strong demand for non- chromatographic alternatives in qualitative / quantitative assessment of the reference compounds. Quantitative NMR [17] is a versatile tool used for simultaneous detection and quantification of the compounds along with impurities. NMR involves direct determination and is non destructive in nature. It requires fewer cleanups as compared to HPLC and HPLC-MS and NMR signals are visible even in the complex matrices. ^{1}H-NMR, being more sensitive than ^{13}C-NMR is therefore, more versatile for routine analysis with respect to time and cost. The quantitative NMR concept is being developed with a focus on its potential in the quantification and quality control of the reference compounds. The quantitative – NMR concept offers a way to assess the purity of the natural product in a single analytical step, without the need of performing multiple analyses, while still offering the option to retain the substance.

Many times NMR information is used simply for qualitative analyses, but the desire to obtain quantitative information from NMR is growing rapidly, especially for high throughput techniques like combinatorial chemistry. This seems to arise from the difficulties encountered in performing accurate quantification with mass spectrometric techniques, especially for samples still bound to the resin beads during the solid phase organic synthesis. NMR data can be very quantitative as long

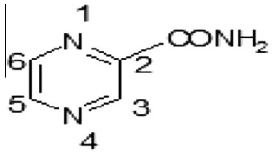

Figure 2 Pyrazinamide.

as certain precautions are observed. Primary importance is the need to ensure that complete relaxation (T_1) has taken place, but the other important considerations include the need to use broad band excitation, to generate flat baselines, to minimize spectral overlap and nOe interferences. Internal standards are also an important aspect of quantification. There appears to be no one perfect internal standard. Some standards are too volatile to give reliable signals (for example TMS) while other internal standards are too involatile to be removed from the sample after measurement (like TSP). Standards have been shown to interact at times with the glass sample container. A good standard should exhibit minimum spectral overlap with the signals of interest. In this article, we report a direct determination and quantitation of pinitol in *A. roseum* extract by means of its NMR signal using pyrazinamide as internal standard.

2. Experimental

2.1. Plant material

The plant material was collected from the hilly terrains of the Jammu region of Jammu and the Kashmir (India) State. The specimen voucher of the plant was deposited in the Herbarium division of the institute.

2.2. Solvents

All the solvents, such as pyridine-d_5, methanol-d_4 and D_2O used for identification as well as for quantification were from Sigma–Aldrich, Bangalore (India), whereas HPLC grade Methanol for ESI-MS analyses (Rankem make) was from Ranbaxy chemicals Ltd. (Mohali, Punjab, India). Pyrazinamide was received as a gift from a private Indian based pharmaceutical company.

2.3. Isolation and characterization of pinitol

Dried and coarsely powdered plant material (500 g) was kept in 95% ethanol (2 L) in a percolator for 15 h at ambient temperature. The solvent was drained off, and the process was repeated four times. All the drained off portions (4×2 L) were combined, filtered and finally evaporated under diminished pressure to yield a residue (36 g). The residue was triturated successively with hexane (4×500 ml) to remove the non-polar part of the extract. The residue (26 g) left behind after trituration was subjected to column chromatography on silica gel (520 g, 60–120 mesh) using ethyl acetate as the eluting solvent. The polarity of the mobile phase was increased by gradual addition of methanol. The fractions, eluted in ethyl acetate: methanol (1:1), exhibited a similar pattern on TLC, were pooled and evaporated under vacuo to yield residue (9.5 g). The residue was crystallized in hot ethanol to yield 5.2 g of pure compound that was analysed for $C_7H_{14}O_6$, m.p. 184–185 °C $[\alpha] = +61.5$ (C: 0.27, H_2O), ^{1}H-NMR (400 MHz Fig. 3), pyridine-d_5: δ 4.80–4.85 (4H, m, H-2, H-3, H-4, and H-5) 4.18–4.23 (1H, t, $J = 6.0$ Hz, H-1), 4.65–4.71 (1H, t, $J = 6.0$ Hz, H-6), 3.97 (3H, s, OMe). When ^{1}H NMR was taken in D_2O, MeOD and DMSO the signal resonating for methoxyl protons of pinitol was observed at δ 3.46, 3.51 and