Spectrophotometric Studies of Spermine Isolated from the Nutmeg of *Myristica fragrans*

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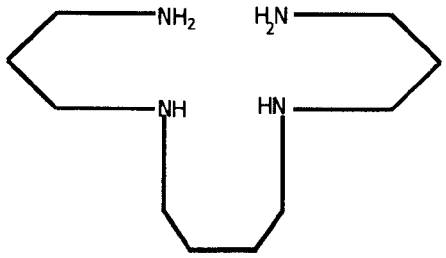
Summary: The seeds of *Myristica fragrans* (Myristicaceae) were studied for spermine. The fruit kernels (nutmegs) were subjected to chloroform extraction. Spermine was isolated by TLC using different solvent systems and flow injection micro column. Spermine acid adduct was prepared for quantitative determination of spermine. Absorbance was noted in visible region on spectrophotometer. Spermine was found to be 0.0051% in fruit kernels of *M. fragrans*.

Introduction

Myristica fragrans (N.O. Myristicaceae) is a bushy tree with numerous spreading branches. It attains a hight of 30-40 feet. It produces fruits all the year round but most heavily in August and September. The fruit appears on the tree mingled with flowers and bears flowers and fruits at the same time. The orange yellow smooth fruit, when ripe, is one of the most beautiful in nature. The fruits resemble small peaches. The pericarp of the fruit is of fleshy, somewhat firm texture and about a half in thickness. When fruit is ripe the pericarp opens by splitting into two halves and the nut appears. It has a deep brown glossy seed coat or shell. The fruit when dried is the nutmeg of commerce. When dried this shell can easily be cracked to remove the seeds [1-7]. The fruit has aromatic flavor and finds its application as a spice in different culinary and food preparations. The seed has medicinal importance and is used as an essential constituent of different medicine in the local Materia medica for the treatment of various ailments [2-4, 8-10]. S. Park reported anticancerous activity of the seeds of *Myristica fragrans* [11]. Medicinal importance of this plant incited us to further investigate the useful attributes of the plant seed.

Spermine is the novel alkaloid, macrocyclic with general formula, C_{10} H_{26} N_4 and structural formula as;



N,N' bis [3-Aminopropyl] – 1,4- butanediamine [12-16]

The name Spermine comes from the discovery of these compounds in human sperms. The presence of Spermine Phosphate crystals is still used as a part of the legal identification of the suspect stains [17]. They have been isolated from various plants.

Spermines take part in diverse physiological processes. They are growth factors for Cultured mammalian and bacterial cells and function in the stabilization of intact cells. They are wide spread in living cells occurring in association with nucleic acid [17]. Due to their biochemical importance spermine have been topic of research by various workers at different times [18]. The present work was intended to analyse spermine using flow injection analytical method.

Those radiations with wave length ranging from 200-350 nm are called ultraviolet radiations and those having 350-750 nm are the visible radiations. The absorption of these radiations provides a good tool for studying various reactions and their mechanisms. The absorption of UV and visible radiation causes electronic transitions in the atom or molecule and characterizes it as a whole. In the UV region approximately 100 Kcal/mole energy is required to excite the electron and to affect its vibrational and rotational energy. The Spermine-acetaldehyde adduct of yellow brown color. The absorption then occurs in the visible region.The method originally uses the UV regions of spectrum because the sample of Spermine absords radiations in this region of spectrum but its λ_{max} is difficult to measure in the UV region and special quartz cell has to be used and it requires a UV- Spectrophotometer. To avoid such problems, the method was modified to use visible spectral range after forming.

Results and Discussion

The new method for quantitative determination of plant amines was developed. Spermine-acetaldehyde adduct was prepared and studied through spectrophotometer for quantitative determinations. Spermine was calculated to be 0.0051% in fruit of *Myristica fragrans*.

Effect of Acetaldehyde on Spermine- acetaldehyde adduct

In order to observe the quantity of acetaldehyde required for the formation of spermine-acetaldehyde adduct. A series of experiments was carried out. On varying concentration of acetaldehyde keeping spermine and acetic acid constant and results of the spectrophotometric studies it was observed that 2mL acetaldehyde is required per millilitre spermine for the formation of spermine-acetaldehyde adduct.

Effect of acetic acid on spermine-acetaldehyde adduct

The objective of this exercise was to observe the minimum amount of acetic acid required for the formation of adduct. For this purpose the volume of acetic acid was varied in the series of 10mL measuring flasks, keeping spermine and acetaldehyde constant. Absorbance of each reaction mixture was recorded 372nm and it was observed that smaller quantity of acid gave high absorbance for the adduct than a larger one. Therefore, it was recommended to use 20μL of acetic acid for the formation of spermine-acetaldehyde adduct.

Effect of time on spemine-acetaldehyde adduct

The adduct was synthesized at 60-70°C and absorbance was measured at different time intervals. From these observations it was found that 20 minutes time is sufficient for maximum formation of adduct. After one hour it decreases and then becomes constant.

Effect of temperature on spermine-acetaldehyde adduct

To observe the most suitable value of temperature at which the maximum formation of adduct occurs, the adduct was subjected to different temperatures, i.e., 20-90°C. From graph it was clear that a temperature of 60°C is favorable for spermine-acetaldehyde adduct.

Experimental

General

Absorbance was measured on Shimadzu UV-120-01 Spectrophotometer. UV lamp UV-58 (Mineralight Lamp of UVP Upland CA 91786 USA) was used for the detection of TLC spots. The pH was measured on CD 620 WPA (Cambridge UK.)

Collection, extraction and isolation

The fruits of *Myristica fragrans* were collected from Botanical Garden, New Campus, Punjab University, Lahore, Pakistan. Specimens of seeds have been kept in Istitute of Chemistry, University of the Punjab, Lahore. The nutmegs (1Kg) of *M. fragrans* were ground and extracted thrice with chloroform at room temperature. The combined extract was evaporated under reduced pressure to obtain a crude syrup. The total chloroform extract was subjected to TLC over Silica gel successively eluting with 1,4 Dioxane and Phosphate Buffer Saline (50:50); Acetonitrile; Ammonia, Butanol, Ethanol (20:60:20) and Phosphate Buffer Saline (PBS) in increasing polarity order. The combined Ammonia, Butanol, Ethanol (20:60:20) fractions were free of solvent and the residue was subjected to flow injection micro-column over silica gel using a mixture of Ammonia, Butanol, Ethanol (20:60:20) in increasing polarity order. The fraction which eluted

with Ammonia, Butanol, Ethanol (20:60:20) was re-chromatographed over silica gel. The fraction obtained provided the semi-solid mass containing the spermine.

Synthesis of spermine-acetaldehyde adduct

Acetaldehyde (3mL) was mixed with spermine (100ppm, 1mL) in a 10mL measuring flask and added Acetic acid (20µL). The reaction flask was whirled for a while and volume was made up to 10mL with chloroform. The flask was heated up to 60-70°C on a water bath for 15 minutes.

Separation of spermine acetaldhyde adduct

TLC plates were spotted with Acetaldehyde, Spermine and their adduct separately using capillary jets and developed in different solvent systems as shown in the Table (1). The UV lamp was used for the location of spots. Spots of spermine and acetaldehyde could not be observed on the developed TLC plates. Only adduct spots were observed and R_f value was calculated (Table 1).

Table 1 Different Solvent Systems used TLC Plates and R_f values for Adduct

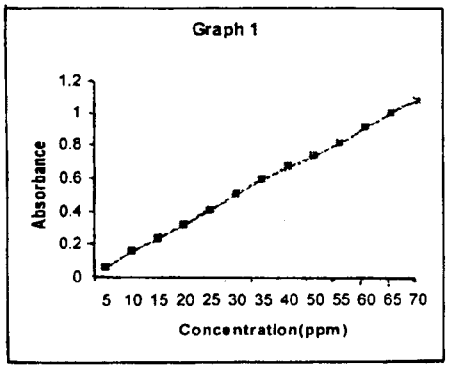
Sr. No.	Solvent System	Ratio	R_f Value for Adduct
1	1,4- Dioxane & PBS	50:50	0.57
2	Acetonitrile	-	0.96
3	Ammonia, Butanol & Ethanol	20:60:20	0.73
4	Phosphate Buffer Saline (PBS)	-	0.66

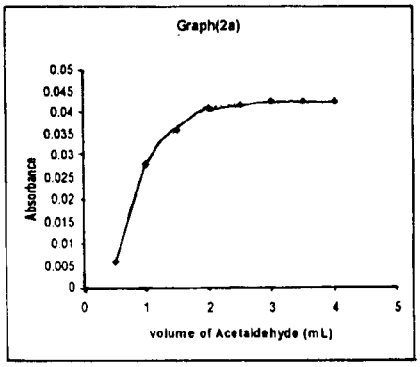
Separation of spermine-acetaldehyde adduct on a microcolumn

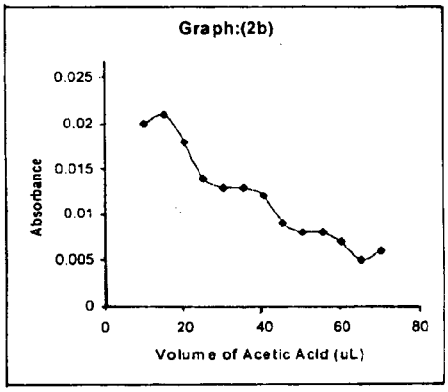
The spermine- acetaldehyde adduct was injected into a mirrocolumn incorporated into a flow injection manifold using ammonia, n-butanol and rectified spirit (20:60:20) as solvents. Separation of adduct was confirmed through flow injection profile and IR-spectrum used for the blank and the sample readings.

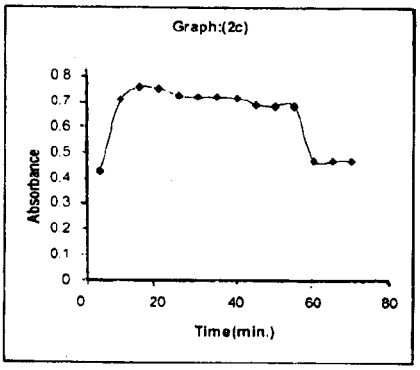
Calibration curves

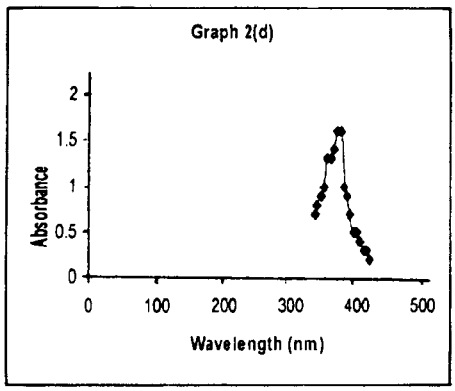
The standard solution of spermine, i.e. 5 to 70 ppm were prepared from its 100 ppm stock solution. To each solution acetaldehyde (3mL) and acetic acid (20µL) were added. The final volume was made up to 10 mL with chloroform. Each solution was heated upto 60 to 70°C on water bath for 15 minutes. Absorbance of each solution was measured. Similar











conditions were used for the blank and sample readings. Results are shown in graph (1).

Effect of acetaldehyde and acetic acid concentrations and variations in time and temperature

The concentration of acetaldehyde required for the formation of spermine- acetaldehyde adduct was varied keeping all other conditions constant. Similar method was adopted for acetic acid concentration variations and effect of time and temperature. Results have been shown in graphs 2a, 2c and 2d.

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