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Potential benefits of triethylamine as n-electron donor in the estimation of forskolin by electronic absorption and emission spectroscopy

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ABSTRACT

Diterpenoid forskolin was isolated from *Coleus forskolii*. The electronic absorption and emission studies of forskolin were investigated in various solvents with an aim to improve its detection limits. The two chromophores present in the diterpenoid are not conjugated leading to the poor absorption and emission of UV light. The absorption and fluorescence spectra were solvent specific. In the presence of a monodentate ligand, triethylamine the detection of forskolin is improved by 3.63 times in ethanol with the fluorescence method and 3.36 times in DMSO by the absorption spectral method. The longer wavelength absorption maximum is blue shifted while the lower energy fluorescence maximum is red shifted in the presence of triethylamine. From the wavelength of fluorescence maxima of the exciplex formed between excited forskolin and triethylamine it is concluded that the order of reactivity of hydroxyl groups in the excited state forskolin is in the reverse order to that of the order of the reactivity of hydroxyl groups in its ground state.

1. Introduction

Forskolin is an oxygenated tricyclic labdane group of diterpenoid. It is isolated from *Coleus forskolii* [1]. The right ring of the decalin portion of forskolin has a structural similarity with α-D-galactose. As a result, forskolin binds to the glucose transporter membrane protein to activate adenylcyclase (AC). Hence it has been an important molecule for studying the physiological role of AC [2–5]. Forskolin leads transient cessation of DNA synthesis [6]. In addition to S phase, it also inhibits progression of cells through other phases of the cell cycle. It induces cell cycle arrest at $G_{0/1}$ phase in human fibroblasts and B lymphoid cells, and has been reported to cause down regulation of *c-myc* mRNA [7]. Forskolin also induces apoptosis in malignant glioma cells and primary granulose cells via up regulation of the cAMP/PKA pathway [8,9]. It showed excellent pharmacological properties such as positive inotropic, antihypertensive, blood pressure lowering, intraocular pressure lowering etc. [10–14]. In spite of innumerable physiological roles, the solubility of forskolin in water is a severe constraint to develop it in to a drug. It is soluble in water only up to 0.001%. Therefore special efforts are made upon to enhance its solubilization in water. The two methods adopted for improving its water solubility are chemical manipulation and physicochemical techniques. In the former, organic synthesis targets not only aqueous solubility but also the selectivity of forskolin, its congeners, analogues and derivatives towards different adenylcyclases isoforms. Alternatively, physicochemical techniques have been employed for enhancing the water solubility of underivatized drug. Notable techniques include micellar solubilization and complexation of forskolin with a host of molecules like cyclodextrins. As the solubility of forskolin in water is negligible a more sensitive technique is required for its assay. The amount of forskolin in the physiologically acceptable media is determined by HPLC. Forskolin was quantitated by high performance liquid chromatography and TLC [15–19] and by ELISA method [20]. However there are very few reports on the quantitative detection of forskolin and therefore there is a need to develop more sensitive tool for its assay. Hence in this paper we report the electronic absorption and emission studies of forskolin with a view to improve its detection limit by adopting physicochemical method.

2. Experimental

2.1. Isolation of forskolin

Isolation and purification of forskolin were carried following Reddy et al. [21]. The structure of forskolin is given in Fig. 1. 500 g of 20% coleus forskolin enriched plant powder extract (purchased from Alkali metal, secunderabad) was dissolved in 1 L of chloroform:methanol (8:2) and adsorbed on to 500 g of silica gel (60–120 mesh) in a glass bowl by constant stirring. The solvent was evaporated at room temperature in two days with constant stirring. The column was dry packed with 2 kg of silica (60–120 mesh) and was eluted with 10 L 100% pet. ether to elute some gummy impurities. Then the polarity was increased by mixing ethyl acetate. 742 fractions each 1 L were collected and examined by TLC keeping HPLC grade forskolin (Sigma Aldrich) as reference. The eluted fractions were collected with different impurities. Pure forskolin compound was obtained with 12% ethyl acetate and 88% pet. ether. The

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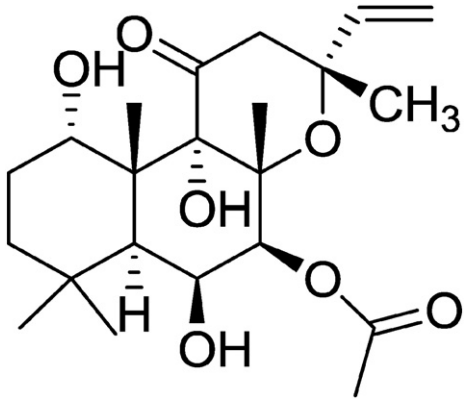

Fig. 1. Structure of forskolin.

impurities got decreased gradually by 150–200, 220–250 and 280–350 elution fractions. The fractions 352 to 460 contained less impurity. Forskolin was eluted in fractions 461 to 562 with a total yield of 85 g of pure forskolin and 10 g of impure forskolin. 85 g of pure forskolin was recrystallized from ethyl acetate and pet. ether (5:95) to get pure compound. The crystals were stirred with *n*-hexane (3 × 15 mL) to remove hexane soluble impurities, and *n*-hexane was removed by filtration. After drying the residue, it was dissolved in 5 mL of chloroform. To this solution, 50 mL of *n*-hexane was added with continuous stirring to remove most of the colouring matter and crude forskolin was obtained as a pale yellow precipitate. The pale yellow solid was dissolved in minimum amount of ethyl acetate to precipitate out colourless pure forskolin by the gradual addition of benzene with continuous stirring. This crystallization step was repeated to get pure forskolin as white powder. The purity of the compound is 95% as shown in its HPLC Chromatogram (Fig. 2).

2.2. Materials

All the solvents employed were of BDH spectroscopic grade and used without any further purification. HPLC grade forskolin was purchased from Sigma Aldrich and used without further purification. 20% coleus forskolin enriched plant powder extract was purchased from Alkali metal, secunderabad.

2.3. Instruments

HPLC was recorded on Agilent 1100 HPLC system, UV–vis spectra were recorded on Elico SL 159 UV–vis spectrophotometer. Fluorescence was investigated on RF-5301PC Shimadzu spectrofluorophotometer with 5 nm excitation and emission slit widths at 18 °C.

3. Results & discussion

3.1. UV–vis studies

Electronic absorption spectroscopy is a widely used analytical method for the assay of various analytes and metabolites. The sensitivity of an assay by the electronic absorption spectral method relies on the chromophoric system present in an analyte in the absence of steric or field effects. Forskolin is a tricyclic diterpenoid with a molecular formula $C_{22}H_{34}O_7$. The chromophores present in the molecule are carbonyl and vinyl groups. These are at isolated positions and are not conjugated. The electronic absorption spectra of forskolin in ethanol are shown in Fig. 3.

From Fig. 3 it can be observed that forskolin exhibited two weak absorption maxima at 224 nm and 308 nm with the molar extinction coefficients of 7.29×10^2 mol^{-1} dm^2 and 0.51×10^2 mol^{-1} dm^2 respectively. Similar less intense weak absorption maxima at ~225 nm and ~300 nm were obtained in other solvents. The absorption band at 225 nm is assigned to ππ* transition while the maximum at 300 nm due to nπ* transition. The nπ* transition is a weak symmetry forbidden transition while that of 225 nm band is an allowed transition. In spite of being allowed, the transition at 225 nm is weak. It is due to its isolated nature i.e., isolated double bonds do not give strong absorption bands. The lower energy absorption maxima of forskolin in the solvents investigated are tabulated in Table 1. A closer inspection of Table 1 reveals that in all the solvents employed there are no significant spectral shifts occurred. The lower energy absorption maximum is varying between 301 nm and 312 nm in the solvents employed and a very little altered intensity of the absorption maxima. However, the intensity varies linearly with the concentration of forskolin as shown in Fig. 4.

A narrow range of linearity between the intensity of the absorption band and the concentration of forskolin limits the use of UV–visible spectroscopy as an analytical tool for its estimation [22,23]. The ππ* transition occurs in the region where the instrument and many solvents

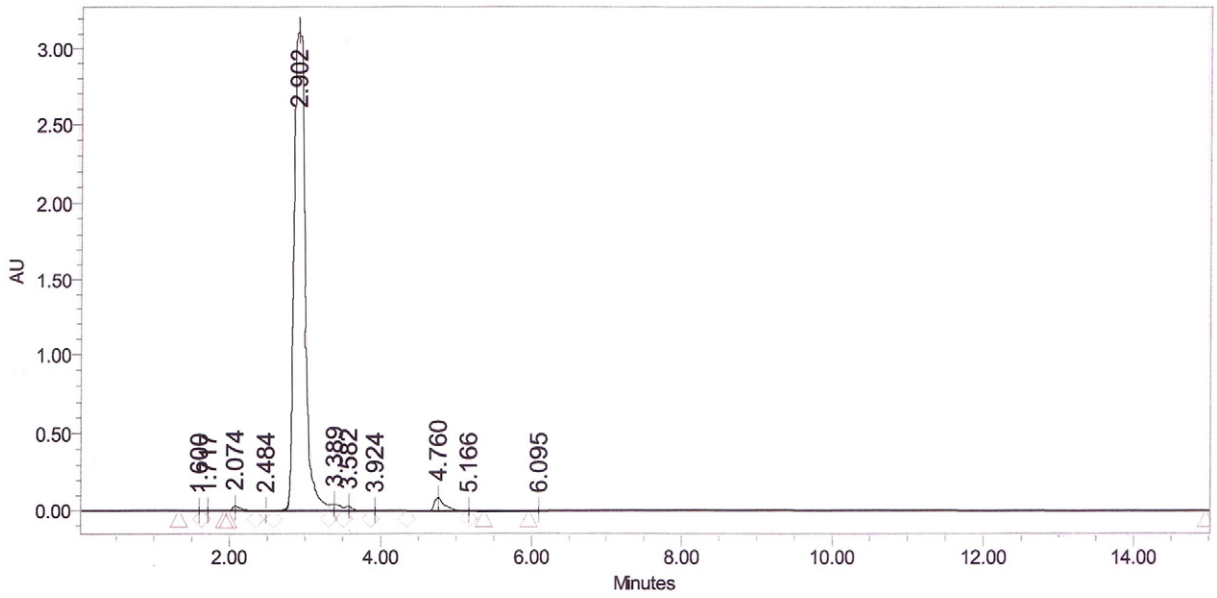

Fig. 2. HPLC Chromatogram of forskolin. (Isocratic system) Solvents acetonitrile:methanol:water (50:10:40), flow rate: 1 mL/min, injection volume 20 μL, Temperature: 40 °C, stop time: 15 min, wavelength: 220 nm.