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Isolation of forskolin from stem of *Coleus forskohlii*

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Abstract
Forskolin was isolated from the stem of *Coleus forskohlii*. Chloroform extract of the powdered stem was subjected to silica gel column chromatography to obtain crude forskolin. The crude forskolin was crystallized using ethyl acetate and *n*-hexane to obtain pure forskolin. The forskolin isolated was characterized by using UV, IR, ESI-Mass, ^{1}H and ^{13}C NMR. The yield was 0.103% w/w based on the dry weight of stem powder. The present study reconfirmed existing data on physical and spectroscopic characterization of forskolin.

Keywords: Coleus forskohlii; Stem; Forskolin; Isolation; Purification; NMR

INTRODUCTION

Coleus forskohlii Briq. (Lamiaceae) grows wild in various parts of India; the roots have long been used in ayurvedic medicine for treating heart and lung disease, intestinal spasms, insomnia and convulsions (1). Forskolin (Fig. 1), a labdane diterpene (7β-Acetoxy-8,13-epoxy-1α,6β,9α-trihydroxy-labd-14-ene-11-one) isolated from *Coleus forskohlii* (2, 3), was observed to activate adenyl cyclases resulting in an increase in cAMP (4). The mechanisms of interaction of forskolin with adenyl cyclases and activation of the latter by forskolin were studied in detail (5-9). Forskolin showed positive effects against a wide range of conditions such as asthma (10), glaucoma (11), hypertension (12), cancer (13), heart disease (14), diabetes (15) and obesity (16). It also showed inhibition of platelet activating factor (17), increase in the rate of sensory nerve regeneration in freeze-lesioned sciatic nerves (18), stimulation of water and cation permeability in aquaporin 1 water channels (19) and direct alteration of gating of a single class of voltage-dependent potassium channels from a clonal pheochromocytoma (PC12) cell line independent of adenylate cyclase activation (20).

Forskolin is widely used in various biochemical studies related to cAMP and adenyl cyclase pathways (21). Forskolin was detected in the stems of *C. forskohlii* by HPLC analysis in the earlier studies published (22, 23). However, to our knowledge, isolation and characterization of forskolin from stem of *C. forskohlii* is not reported so far. This prompted us to isolate and characterize forskolin from stems of *C. forskohlii* and the same is reported here.

MATERIALS AND METHODS

Plant material and chemicals

Dried stems of *C. forskohlii* were purchased from an herbal medicine vendor (Khandige Herbs and Plantations Pvt. Ltd., Poonamallee High Road, Arumbakkam, Chennai, India) in Chennai, India and a voucher specimen was deposited in Department of Pharmacognosy, C.L. Baid Metha College of Pharmacy, Chennai, India. All the other chemicals used were of analytical grade and purchased from S.D. Fine chemicals, Mumbai, India.

Apparatus and general experimental procedure

Thin layer chromatography was performed with aluminium backed precoated plates of silica gel $60F_{254}$ (E. Merck, Darmstadt, Germany) in toluene:ethyl acetate (80:20, v/v) as mobile phase. Anisaldehyde-sulphuric acid was used as the spray reagent. Melting point was checked with Toshniwal melting point apparatus (Mumbai, India) with 2 mg of sample in a melting point capillary tube. UV spectrum was recorded on Hitachi U-2001 Spectrometer (Tokyo, Japan). 0.8 mg of sample was dissolved in few ml of methanol by gentle shaking and the final volume was made upto 10 ml with methanol in a volumetric flask. This solution was used for recording the UV spectrum against a solvent methanol blank. IR spectrum was recorded on Perkin Elmer Model Paragon 1000 FT-IR spectrometer (CT, USA) in KBr disc. 1 mg of sample was mixed with 50 mg of KBr salt and a translucent disc was made by pressing this mixture in a die. This disc was used for recording the IR spectrum. Mass spectrum was obtained from esquire 2000 ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with ESI interface in positive mode. 1 mg of sample was dissolved in few ml of methanol by gentle shaking and the final volume was made upto 10 ml in a volumetric flask. This solution was used for recording the mass spectrum. NMR spectra were recorded in $CDCl_3$ (Cambridge Isotope Laboratories, Inc., MA, USA) using Avance DPX 200

spectrometer (200 MHz for ^1H and 50 MHz for ^{13}C) (Bruker BioSpin GmbH, Rheinstetten, Germany) using residual solvent peak as the reference standard. 15 mg of sample was dissolved in 0.4 ml of $CDCl_3$ by gentle shaking for 10 minutes and used for NMR analysis.

Extraction and isolation

500 g of powdered stem material (particle size 100-1000µm obtained by sieving) was extracted with chloroform (3 X 500ml) under continuous stirring at room temperature (30ºC ± 5ºC) for 2 h in each extraction. The chloroform extract was filtered and concentrated to dryness under reduced pressure (474 mbar) at 40ºC to obtain 10.3 g of semisolid residue. A portion of this residue (2 g) was chromatographed on 60 g of silica gel (230-400 mesh) packed as a slurry in toluene in a column (100 cm X 2.5 cm i.d.). It was eluted with toluene (100%), toluene:EtOAc (95:5), toluene:EtOAc (90:10), toluene:EtOAc (85:15) and toluene:EtOAc (80:20). Fractions of toluene:EtOAc (80:20) were concentrated under reduced pressure (240-77 mbar) at 40ºC to obtain a solid residue (500 mg). This solid residue on repeated crystallization with EtOAc:*n*-hexane (1:15, v/v) yielded forskolin (100 mg).

RESULTS

Forskolin, off-white powder (yield – 0.103% w/w), crystallized from ethyl acetate and *n*-hexane (1:15), TLC R_f 0.27 (toluene and ethyl acetate, 8:2, v/v, anisaldehyde-sulphuric acid spray reagent), m.p. 230-232 ºC, UV (MeOH) λ_{max} 199 nm (log □□□□□□), FT-IR □$_{max}$ (KBr) 3445, 3220, 2923, 1699, 1375, 1273, 1113, 1055, 769 cm-1, ESI-MS, *m/z* 433.24 $[M+Na]^+$. For ^1H and ^{13}C NMR data see Table 1.

DISCUSSION

Extraction with chloroform at room temperature was carried out three times to extract forskolin completely as much as possible. Solvents for crystallization were selected based on the solubility and polarity of forskolin and the associated impurities and also based on the literature (2). Thin layer chromatography was performed to monitor the extraction, separation, isolation and crystallization processes. Melting point was found to be 230-232 ºC and complying with the data given in the literature (2). UV spectrum of sample in methanol showed λ_{max} at 199 nm in a peak ranging from 195-233 nm. FT-IR spectrum showed □$_{max}$ peaks at 3445 and 3220 $cm^{-1}$for OH groups, 2923 cm^{-1} for free methyl groups, 1699 cm^{-1} for carbonyl groups, 1375 cm^{-1} for two methyl groups attached at quarternary carbon (C-4), 1273 cm^{-1} for ester group, 1113 and 1055 cm^{-1} for C-O-C linkage between C-8 and C-13 and C-O-C linkage between C-7 and its O-acetyl group and 769 cm^{-1} for CH_2 groups. The ESI-MS spectrum showed a peak at *m/z* 433.24 $[M+Na]^+$corresponding to the molecular formula $C_{22}H_{34}O_7$ with molecular weight 410.23.

^1H NMR spectrum showed six singlet signals at 1.00 ppm (CH_3, position 18), 1.23 ppm (CH_3, 19), 1.31 ppm (CH_3, 16), 1.41 ppm (CH_3, 20), 1.68 ppm (CH_3, 17) and 2.14 ppm (CH_3, 22) for five free methyl groups and one acetyl group at 2.14 ppm (CH_3CO, 22), each corresponding to three protons. Two doublets at 4.95 ppm and 5.27 ppm indicated the cis and trans protons of exomethylene group (H-15) of labdane diterpene moiety. One doublet of doublets at 5.91 ppm with trans coupling (*J* = 10.5 Hz) indicated the olefinic proton (H-14). Two down field shifted doublets at 3.17 ppm and 2.45 ppm with geminal coupling (*J* = 17 Hz) indicated a diastereotopic protons of a methylene group adjacent to a carbonyl group (H-12). One doublet at 5.45 ppm (H-7), one doublet at 4.54 ppm (H-1) and one multiplet at 4.43 ppm (H-6) each integrating for one proton, indicated protons adjacent to heteroatoms. Further signals of ^1H NMR spectrum were in accordance with the data published (17). ^{13}C NMR spectrum showed six methyl groups at 19.83 ppm (C-20), 21.14 ppm (C-22), 23.57 ppm (C-17), 24.31 ppm (C-19), 31.54 ppm (C-16) and 32.96 ppm (C-18). DEPT 135 spectra showed four methylene groups at δ 26.59 ppm (C-2), 36.06 ppm (C-3), 48.71 ppm (C-12), and 110.73 ppm (C-15) with highest chemical shift value for the olefinic carbon. Two carbonyl groups were identified at 205.32 ppm (C-11) and 169.65 ppm (C-21). Carbons C-1 (δ 74.43 ppm), C-6 (69.95 ppm) and C-9 (82.63 ppm) were downfield shifted because of the presence of hydroxyl groups. Further signals were in accordance with the data published in the literature (24). Collectively, all these spectroscopical data confirmed the identity and structure of forskolin (Fig. 1).

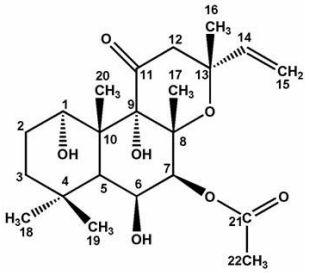
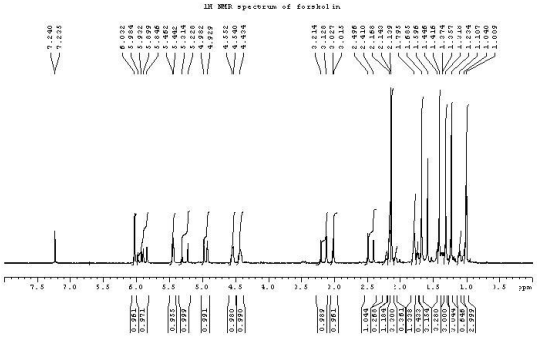
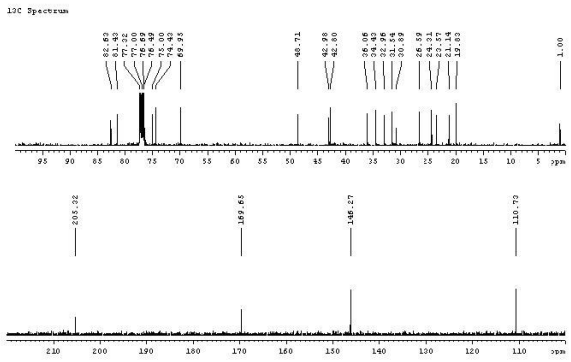
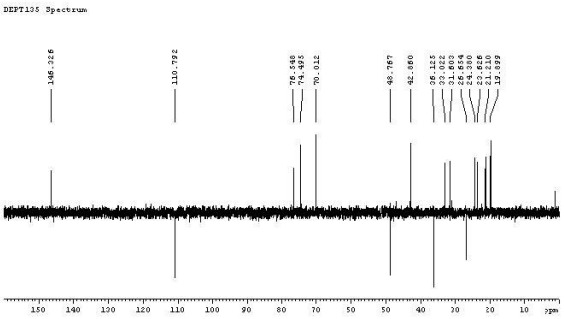


Fig. 1. Structure of forskolin



^{1}H NMR Spectrum of Forskolin





DEPT135 NMR Spectrum of Forskolin

^{13}C NMR Spectrum of Forskolin

Table 1.
^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data of forskolin isolated in $CDCl_3$.

Position	^{1}H Chemical shift mult. (*J*, Hz)	^{13}C Chemical shift
1	4.54 d (2.3)	74.43
2	1.44 m 2.15 m	26.59
3	1.74 m 1.10 m	36.06
4		34.43
5	2.15 m	42.80
6	4.43 m	69.95
7	5.45 d (4.1)	76.49
8		81.43
9		82.63
10		42.98
11		205.32
12	3.17 d (17.0) 2.45 d (17.0)	48.71
13		75.00
14	5.91 dd (10.5, 17.1)	146.27
15	4.95 d (10.5) 5.27 d (17.1)	110.73
16	1.31 s	31.54
17	1.68 s	23.57
18	1.00 s	32.96
19	1.23 s	24.31
20	1.41 s	19.83
21		169.65
22	2.14 s	21.14

In conclusion, forskolin was isolated from stem of C. forskohlii and characterized by UV, IR, ^{1}H and ^{13}C NMR techniques. This study confirmed the reports of the earlier studies (22, 23) about the presence of forskolin in the stem of *C. forskohlii*. Also, it reconfirmed the physical and spectroscopical data of forskolin published earlier (2,3,24).

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