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ISOQUINOLINE ALKALOIDS FROM ETHNO BOTANICALLY IMPORTANT *DICENTRA PAUCINERVIA*

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ABSTRACT

From the tubers of the ethno botanically important medicinal plant *Dicentra paucinervia*, two isoquinoline alkaloids, protopine and allocryptopine were isolated. Structures of the compounds were elucidated by spectroscopic data. Quantitative analysis of protopine in the tubers cultivated in two different areas was also carried out using HPTLC.

Key words: *Dicentra paucinervia*, Protopine, Allocryptopine, HPTLC

INTRODUCTION

Dicentra Bernh. (Family: Fumariaceae), is a genus of herbaceous flowering plants native to Asia and North America[1]. The genus is reported to have twenty species[2]. A large number of physiologically active isoquinoline alkaloids have been isolated from the tubers of many species of *Dicentra* and are classified according to their structures as aporphines, protpberberines, protopines and cularines alkaloids[3-6]. Protopine has been found to possess a range of pharmacological properties: anti-acetylcholinesterase, anti-amnesic, phospholipase and thromboxane synthetase inhibitory, weak spasmolytic, weak anti tumor, smooth muscle stimulate, bactericidal and sedative[6-8]. *Dicentra paucinervia* distributed in North- Eastern states, Arunachal Pradesh, Sikkim, West Bengal, Meghalaya, Nagaland, of India, is a highly potent ethno medicinal herb. The tuberous roots are perennial in nature and tuber is the planting material for the plant. The tubers have been used for years by section of Naga ethnic tribal communities living in eastern Nagaland and adjoining Manipur state in the treatment against various diseases like diabetics, malaria, typhoid and

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other common fevers, pneumonia, diarrhoea, dysentery, carminatives/ flatulence, stomach disorders, cut/ injury etc. The plant is cultivated on small scale by these communities for their medicinal uses. Yield of the tubers is estimated to be about 4800 Kg/acre/ annum[9]. To the best of our knowledge, chemistry of this plant species has not been examined previously. Prompted by above facts, the present study was aimed to examine the chemistry of tubers of *Dicentra paucinervia* in order to isolate and characterize constituents responsible for its biological activity. It was also aimed to develop a HPTLC method for quantification of protopine in the tubers. The plant has also been grown in nursery of Non Wood Forest Products Division of our institute for its possible utilization in the Uttarakhand state having agro climatic conditions different from Manipur (Table 1). Therefore, HPTLC method was also applied for comparative evaluation of protopine in tubers grown in the two different areas.

Table 1: Agro-climatic conditions of Manipur and Dehra Dun

Conditions	Manipur	Dehra Dun
Elevation (msl, m)	1750	640
Temp. (min.) °C	0	1
Temp. (max.) °C	30	37
Average rainfall (mm)	2250	1800
Soil type	Clay Loam	Loam

EXPERIMENTAL

All the melting points are uncorrected. IR spectra in KBr were recorded on a Shimadzu FT-IR 8400 Spectrometer. UV spectra were measured on a Chemito 2500 UV-VIS Spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 MHz spectrometer in $CDCl_3$ with TMS as internal standard. Chemical shifts are reported in δ (ppm). The EIMS were recorded on a Waters Micromass Q-Tof Micro. mass spectrometer. All solvents used were of laboratory reagent grade. For HPTLC analysis, solvents used were of HPLC grade. Silica gel (Sd Fine) of 100-200 mesh was used for column chromatography and zones on TLC (silica gel G) were detected using the Dragendorff's reagent. HPTLC analysis was carried out on a CAMAG HPTLC system using following conditions.

Standard solution

10 mg of protopine was dissolved in 10 mL of $CHCl_3$ and diluted with $CHCl_3$ 1 : 20 for analysis

Layer

TLC plate silica gel 60F254Merck, 10 x 10 cm.

Sample application

6 mm bands using the automatic TLC sampler 4 equipped with 100 µL syringe, application volume 2-10 µL for samples and standards, track distance 10.0 mm, first application position 14.5-14.9 mm and distance from the lower edge of plate 6.2 – 7.4 mm .

Chromatography

In twin trough chamber with the mobile phase 25% MeOH : $CHCl_3$

Evaluation

Densitometer evaluation of the plates was performed by measuring absorbance at λ290 nm using a TLC scanner 3 with D_2 lamp, slit size 5 x 0.45 mm in conjunction with WinCATS software for quantitation**.** A typical densitogram obtained for pure protopine by use of this procedure is shown in Fig. 1. Calibration curve of protopine was established by employing 4 level calibration (n = 2) from 300-450 ng / band with polynomial calibration via peak height. For quantitative analysis of protopine in the tubers, 5 mg of methanolic extract was dissolved in 1 mL of methanol and used as such for analysis. Sample (extract) solution 2 µL (n = 2) and standard solution (4-9 µL) were applied to a chromatographic plate and the area of the protopine peak was measured. The amount of protopine present in the sample solution was then determined from peak area values from the standard and the sample solution using

Plant material

Tubers of *Dicentra paucinervia* were collected from Manipur and Dehra Dun. The plant was identified by Dr. H.B. Naithani, Systematic Botanist, Botany Division, Forest Research Institute, Dehra Dun by comparing our specimen with authentic specimen preserved in the herbarium of the institute.

Extraction and isolation

Air dried and powdered tubers (600 g) were extracted sequentially with hot petroleum ether (60-80ºC), chloroform and methanol (l x 3) till complete extraction. Removal of solvent on a water bath yielded 4.4 g, 75.6 g and 98.4 g extracts, respectively. The methanol extract (90 g) was acidified with 0.1N HCl solution to pH 4. The acidic mixture was then treated with $CHCl_3$ and the chloroform soluble fraction was discarded, basified to pH 9 using NH_4 OH and extracted with $CHCl_3$. Removal of solvent on water bath afforded total crude alkaloids (11 g,), which was chromatographed over silica gel, eluting with MeOH : $CHCl_3$ (2 : 98 to 10 : 90) to afford two pure compounds - protopine (3 g) and allocryptopine (2 g).

Protopine (I): White crystalline

MP: 208 ºC.

R_f : 0.7 ($CHCl_3$ -MeOH, 3 : 1)

IR (KBr): 3040, 1672 and 1083 cm^{-1}.

UV λ_{max} ($CHCl_3$) nm: 249.8 and 290.8

^{1}H NMR (400 MHz, $CDCl_3$): 1.90 (3H, s, H-7), 2.52 (2H, bs, H-5), 3.56 (2H, s,H-8), 3.77 (2H, bs, H-13), 3.43 (2H, s, H-6), 5.90 (2H, s, H-15), 5.93 (2H, s, H-16), 6.65-6.67 (1H, d, H-12), 6.63 (1H, bs, H-4), 6.68 (1H, s, H-11), 6.89 (1H, s, H-1) and 6.89 (1H,s, H-11)

^{13}C NMR (400 MHz $CDCl_3$): 31.82 (CH_2), 41.46 (N-CH_3), 46.49 (CH_2), 50.80 (CH_2), 57.82 (CH_2), 100.87 (O-CH_2-O), 101.22 (O-CH_2-O), 106.7 (CH), 108.12 (CH), 110.50 (CH), 117.92 (C), 125.08 (CH), 129.02 (C), 132.82 (C), 136.17 (C), 145.89 (C–O–CH_2–O), 146.01 (C–O–CH_2–O), 146.35 (C–O–CH_2–O), 148.02 (C–O–CH_2–O) and 194.97 (CO).

MS (EI, 70 eV): m/z (%) = 354 $[M + 1]^+$ (100)

Yield: 0.5%

Allocryptopine (II): White crystalline

MP: 154-156^{0}C

R_f: 0.4 ($CHCl_3$ -MeOH, 3 : 1).

IR (KBr): 3010, 2829,1673 and 1079 cm^{-1}.

UV λ_{max} ($CHCl_3$) nm: 255.4 and 282.4

^1H NMR (400 MHz, $CDCl_3$): 1.75 (3H, s, H-7), 2.64 (2H, bs, H-5), 3.22 (2H, bs, H-6), 3.61 (2H, bs, H-8), 3.67 (3H, s, CH_3), 3.71 (3H, s, CH_3), 3.90 (2H, bs, H-13), 5.81 (2H, s, H-15), 6.52 (1H, s, H-4), 6.68-6.70 (1H, d, H-12), 6.79-6.81 (1H, d, H-11) and 6.84 (1H, s, H-1).

^{13}C NMR (400 MHz $CDCl_3$): 32.2 (CH_2), 41.04 (N-CH_3), 46.07 (CH_2), 49.95 (CH_2) 55.46 (CH_2), 57.37 (O-CH_3), 60.58 (O-CH_3), 101.05 (O-CH_2-O), 109.08 (CH), 110.26 (CH), 110.40 (CH), 127.56 (CH), 128.41(C), 129.41 (C), 132.74 (C), 135.81 (C), 145.88 (C-O-CH_2-O), 147.19 (C-O-CH_2-O), 147.88 (C-O-CH_3), 151.41 (C-O-CH_3) and 193.97 (CO).

MS (EI, 70 eV): m/z (%) = 370 $[M + 1]^+$ (100)

Yield: 0.33%

RESULTS AND DISCUSSION

The tubers were extracted sequentially with hot petroleum ether (60-80ºC), chloroform and methanol. Removal of solvents yielded three respective extracts. The methanol extract was acidified and worked up in a usual manner to afford total crude alkaloids. Fractionation of the total alkaloids over silica gel and elution with varying amount of methanol in chloroform afforded two pure compounds. These were isolated as white amorphous solids and crystallized from methanol chloroform. Both gave positive test with Dragendorff's reagent. Their molecular formulas, $C_{20}H_{19}NO_5$ and $C_{21}H_{23}NO_5$, respectively were inferred from their mass spectra. The UV spectrum showed the characteristic peaks at 249.8 and 290.8 mm (for protopine) and 255.4 and 282.4 nm (for allocryptopine). Their IR spectra exhibited bands at 3040/3010, 1672/1673 and 1083/1079 cm^{-1} for presence of aromatic stretching, carbonyl group and methylene dioxy bridge, respectively. A sharp band at 2829 cm^{-1} in the IR spectra of allocryptopine was attributed to the presence of methoxyl group. Four singlets at δ 1.90, 5.90. 5.93 and 6.89 observed in the ^1H NMR spectrum corresponded to the N-CH_3 , H-16 and H-15 of methylene dioxy bridge and H-1 protons of protopine, while presence of N-CH_3, H-15 of methylene dioxy bridge and O-CH_3 in allocryptopine was indicated by singlets at δ 1.75, 5.81, 3.74 and 3.71, respectively.

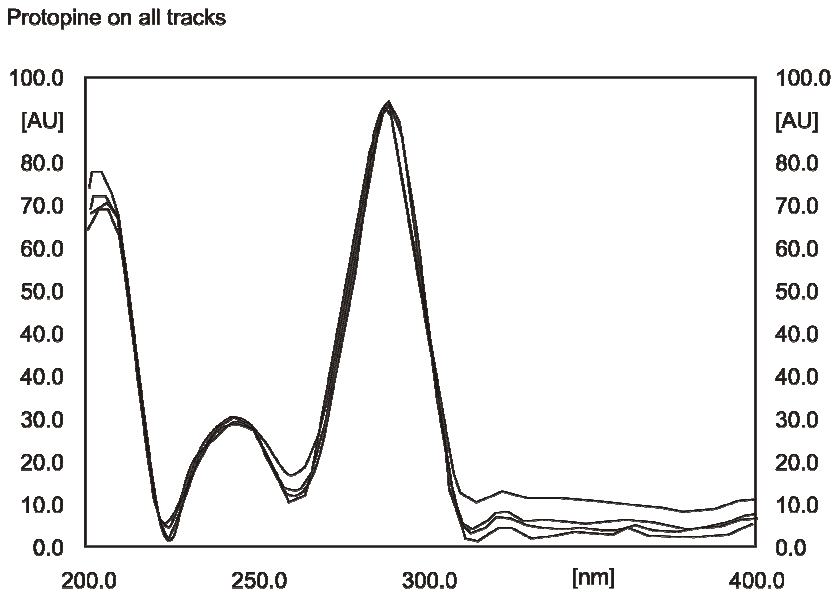


Fig. 1: Densitogram obtained from pure protopine

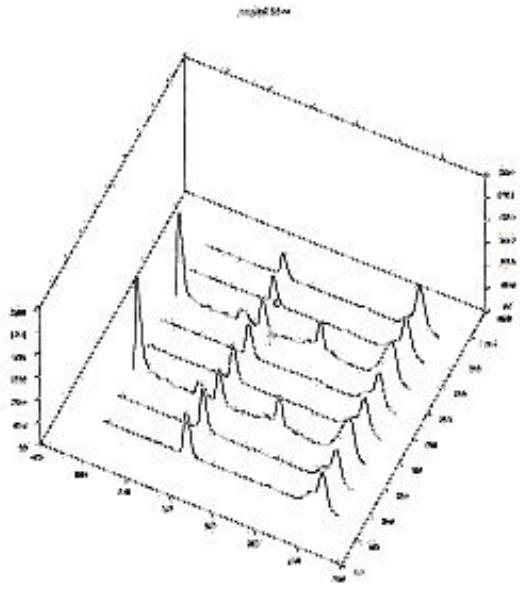


Fig. 2(a)

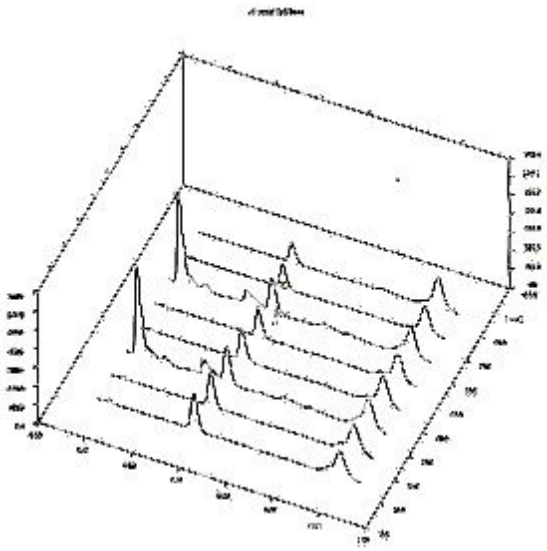


Fig. 2(b)

Fig. 2: 3Dgraphic of chromatograms of extracts (tubers cultivated in Manipur (a) and Dehra Dun (b) (tracks 3 and 6) and standards (tracks 1,2,4,5,7,8)

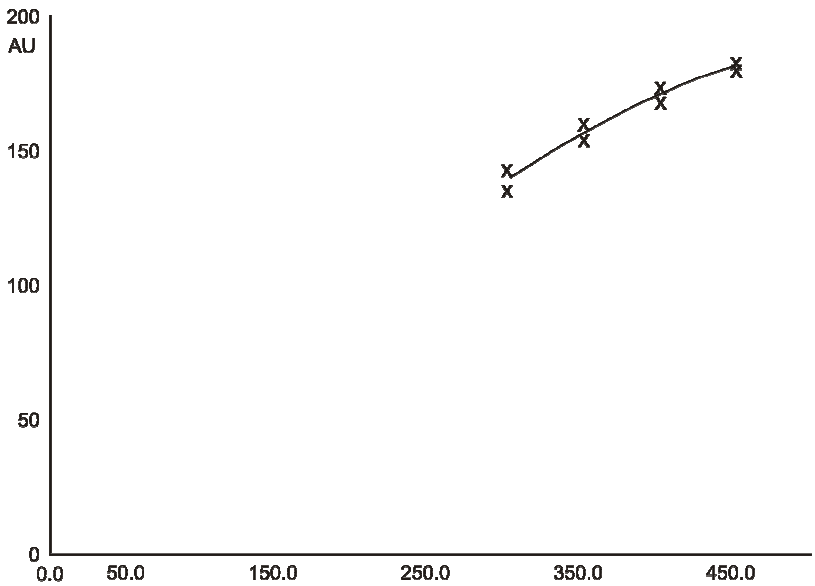
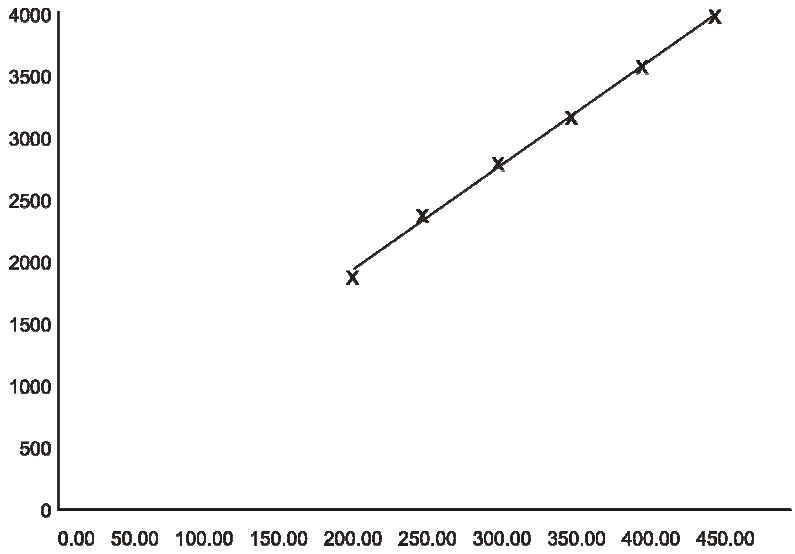
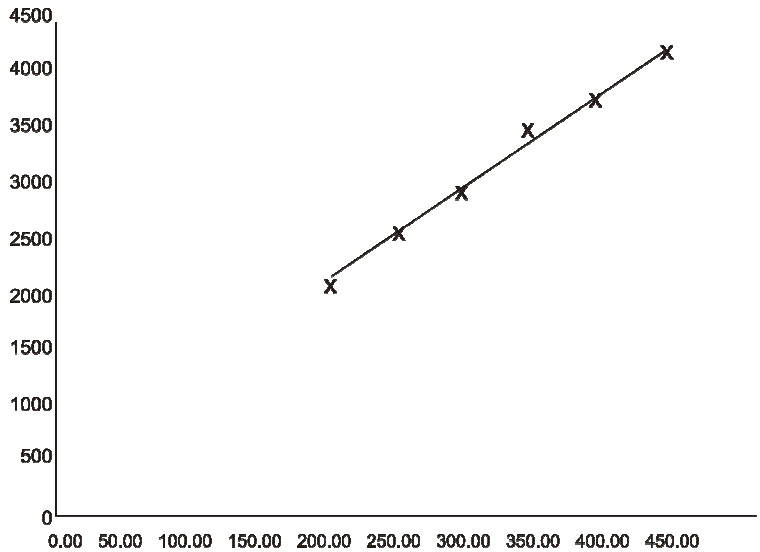


Fig. 3: Calibration curve of protopine



(a)



(b)

Fig. 4: Calibration curve of extracts (tubers cultivated in Manipur (a) and Dehra Dun (b) and protopine

DEPT spectrum showed the presence of one methyl, four methylene, four methine, eight quaternary, one carbonyl and two methylene dioxy bridge carbons for protopine, while signals for one methyl, four methylene, four methine, eight quaternary, one methylene dioxy bridge, one carbonyl and two methoxyl carbons were observed in DEPT spectrum of allocryptopine. These NMR data were in good agreement with these reported in literature[5,10,11]. This is the first report of the occurrence of protopine and allocryptopine in *Dicentra paucinervia*

Regression analysis of calibration data for protopine showed a polynomial regression (regression coefficient, r = 0.9876; standard deviation, sdv 1.96) (Fig. 3), which established the working range of protopine from 300-450 ng / band. As evident from Fig. 2, good separation of protopine in the extract from other constituents was achieved. The linearity curve for the samples and the standard are shown in Fig. 4 (a) and 4(b). Regression analysis of these curves (r = 0.9993; sdv = 1.13% and r = 0.9969; sdv = 2.12%) and also that from the protopine (Fig. 3) indicate that the reproducibility of the method proposed is good. Using the data obtained, the protopine content in the extract and the tubers grown in Manipur and Dehra Dun area was found to be 3.09, 0.51 and 3.88, 0.67%, respectively. It was, therefore, inferred that *Dicentra paucinervia* could possibly be grown in Dehra Dun for its promotion and utilization. This is also the first report on quantitation of protopine in the tubers of *Dicentra paucinervia* by HPTLC.

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