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An isoquinoline alkaloid, Protopine from Corydalis govaniana growing in Himalayan region of Nepal

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Abstract

Chemical investigation of the roots of Corydalis govaniana resulted in the isolation and characterization of one isoquoniline alkaloid, protopine(1). The structure was established on the basis of extensive spectroscopic data analysis and comparison with spectroscopic data reported.

***Key words**: Corydalis govaniana, Fumariaceae, Isoquinoline alkaloid.*

Introduction

Corydalis govaniana Wall (Fumariaceae) is glabrous herb and is distributed in Himalayas of Pakistan, India and in Eastern, Central and Western part of Nepal. It grows in damp and shady places at 2400 to 4800 m attitude[1]. A number of medicinal values have been reported in Indian and Chinese systems of medicine for corydalis species[2-4]. The root of *Corydalis govaniana* is used during diarrhea and dysentery[2]. Previous studies on *Corydalis govaniana* led to the isolation of many isoquinoline alkaloids[5,6,7]. Owing to its unusual geographical location, it was decided to analyze the plants in terms of its alkaloidal content. Our study of a *Corydalis govaniana* has resulted in the isolation and characterization of one isoquinoline alkaloid (1).

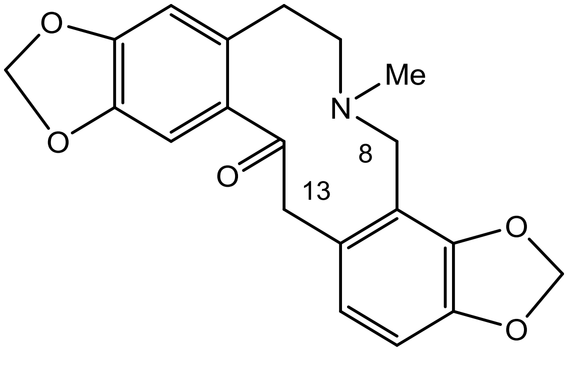

***Figure** 1:* Protopine : 4,6,7,14-Tetrahydro-5-methylbis[1,3]benzodioxolo[4,5-c:5′,6′-g]azecin-13(5H)-one

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Experimental

The melting point was determined on a Toshniwal apparatus and uncorrected UV spectrum was recorded with Perkin-Elmer Lambde spectrophotometer using spectral methanol. An IR spectrum was recorded in KBr pellets. ^{1}HNMR spectra were recorded in 500 MHz in deuterated acetone using tetra methyl silane (TMS) as internal reference. Mass spectrum was performed on JEOL mass spectrometers operating at 70 eV. The purity of substance was checked on TLC plates.

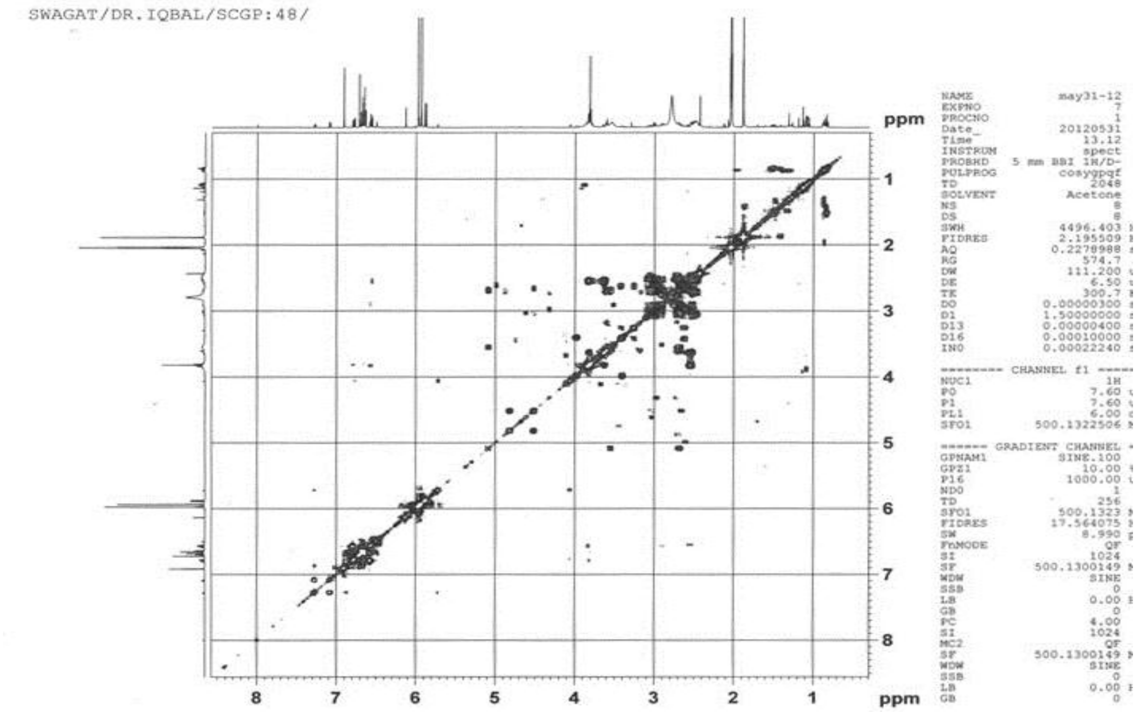
The roots of *Corydalis govaniana* was collected from Langtang, Nepal and identified by comparison with the authentic herbarium specimen at the National Herbarium Laboratory, Kathmandu, Nepal. Air-dried roots of *Corydalis govaniana* (2kg) was extracted with methanol for seven days in cold percolator. After removal of methanol under reduced pressure, the residue (354.30gm) was treated with 7% citric acid and separated to alkaloid fraction according to the procedure of R.N. Jha et.al.[8]

The fraction obtained using above procedure was analyzed on TLC for alkaloids by spraying with Dragendorff's reagent. The chloroform extract (41.12gm) was chromatographied over silica-gel column using solvent of increasing polarity. The eluants from C_6H_6: Acetone (1:1) were mixed and solvent removed to give crude product which was re-chromatographied over silica-gel column to get pure compound which on crystallization from methanol yield 20 mg of compound (**1**).

Compound 1: M.P. 207^o - 208^o C, uv λ max (MeOH) 293 mμ, IR (KBr, cm^{-1}) 1675, ^{1}HNMR (in Table 1), 2D NMR in figure 1(2), ms (m/z,%), 353(10)148(100) (Scheme -1)

Table: 1 *500 MHz ^{1}HNMR spectral data of Compound **1** in Acetone*

Chemical shift	**Proton count**	**Splitting pattern**	**Probable assignment**
2.04-3.83	8H	m	C-5-H2, C-6-H2,C-8-H2, & C-13-H2
5.93	2H	s	CH_2O-
5.97	2H	s	CH_2-O-
6.91	1H	s	C-1-H
6.63	1H	s	C-4-C-H
6.72	1H	d	C-11-H
6.78	1H	d	C-12-H
1.88	3H	s	N-CH_3



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EXPNO                7
PROCNO               1
Date_         20120531
Time             13.12
INSTRUM          spect
PROBHD   5 mm BBI 1H/D-
PULPROG       cosygpqf
TD                2048
SOLVENT        Acetone
NS                   8
DS                   8
SWH           4496.403
FIDRES        2.195509
AQ           0.2278988
RG               574.7
DW             111.200
DE                6.50
TE               300.7
D0          0.00000300
D1          1.50000000
D13         0.00000400
D16         0.00010000
IN0         0.00022240

======== CHANNEL f1 ====
NUC1                1H
P0                7.60
P1                7.60
PL1               6.00
SFO1       500.1322506

====== GRADIENT CHANNEL
GPNAM1        SINE.100
GPZ1             10.00
P16            1000.00
ND0                  1
TD                 256
SFO1          500.1323
FIDRES       17.564075
SW               8.990
FnMODE              QF
SI                1024
SF         500.1300149
WDW               SINE
SSB                  0
LB                0.00
GB                   0
PC                4.00
SI                1024
MC2                 QF
SF         500.1300149
WDW               SINE
SSB                  0
LB                0.00
GB                   0
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Figure 2: *2D NMR/COSY*

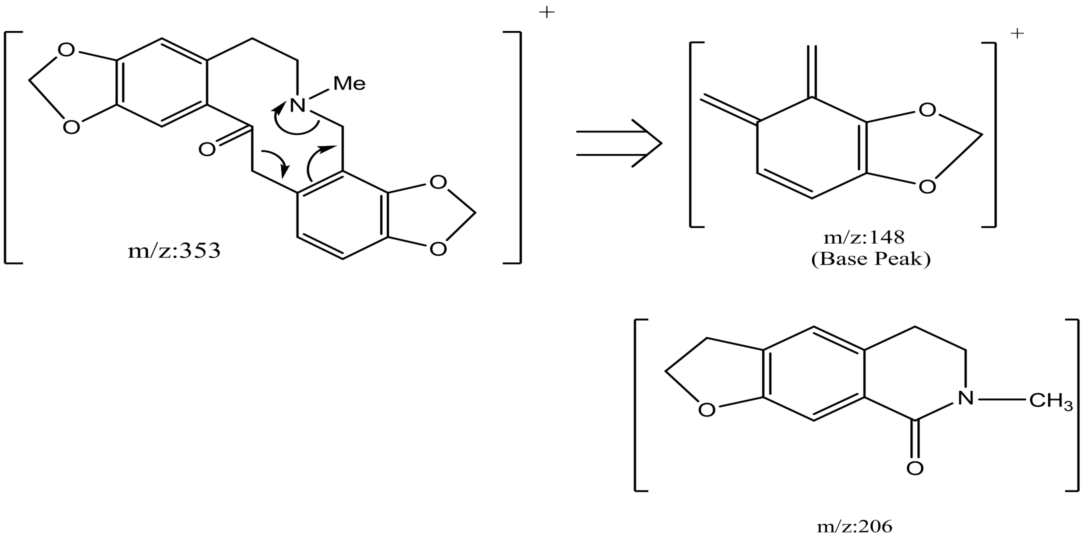
Result and Discussion

The compound (**1**) was obtained as colourless granules. The molecular formula of compound based on the high resolution mass spectrum was found to be $C_{20}H_{19}NO_5$; m/z 353 (M^+), 148 (base peak). A fragmentation pattern indicative of protopine could be easily rationalized (scheme 1). The ultraviolet spectrum in MeOH showed absorption maxima at 293 mµ like that of isoquinoline alkaloids[9]. The IR spectrum contained an absorbance at 1675 cm^{-1} indicating the presence of a trans annular ground state interaction between the carbonyl and the basic nitrogen, so that the carbonyl group in appreciably of the amide type; $0=C\leftarrow N\text{-}CH_3$. The ^{1}HNMR spectrum of 1 (Table-1) showed the following; two methylene dioxy group at δ 5.93 and 5.97 (each 2 H S); two aromatic protons as singlet at δ 6.91 and 6.63; two coupled aromatic protons as doublet at 6.78 and 6.72; one N-methyl group at δ 1.88(3H,S); four aliphatic protons as multiplet at 2.04 – 3-83. Its structure was also favored by 2D NMR. Based on above evidence, the structure of **1** was determined (Fig.1) and named as protopine. Further structure of this compound was confirmed by the comparison with reported data [10,11].

Conclusion

The structure of compound **1** was established by physical, chemical and spectroscopic data. The physical constant and spectroscopic data (IR, UV, NMR, MS) of the base were identical to those of protopine. This is the first report of the occurrence of protopine in *Corydalis govaniana* growing in Himalayan region of Nepal.

Scheme 1



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