
Bioactive Constituents from *Pleurospermum Densiflorum* (“Apiaceae”)

Phool Singh Rajpoot

Professor

Department of Chemistry

Govt. P.G. College, Chharra, Aligarh, U.P. (India)

ABSTRACT

Coumarins, Bergapten, Bergaptol have been isolated from benzene and Chloroform fraction of the *P. densiflorum* and identified by means of ^1H NMR, ^{13}C NMR, I. R. Spectrums, Mass Spectrum data as well as by colour reactions.

Key words –Coumarins, Bergapten, Bergaptol, *P. densiflorum*, Apiaceae, High Altitude Himalayan herbs.

PLANT MATERIAL

The plant *Pleurospermum densiflorum* (Apiaceae) was collected in the month of September at an altitude of 17500-18000 ft. along the snow lines, from Millam glaciers of the Kumaon Himalaya, Uttanchal, India. The plant was identified in the Department of Botany, Kumaon University, Nainital, well as Forest Research Institute, Dehradun.

EXPERIMENTAL

Extraction and isolation - Shade dried aerial parts of *P. densiflorum* were pulverised and 950 gm powder material extracted in Soxhlet apparatus with 90% MeOH for 120 hrs. After complete extraction it was concentrated under reduced pressure in a rotatory vacuum evaporator.

The concentrated MeOH residue was further extracted and fractionated with petroleum ether (60-80°C), benzene (78-81°C), chloroform (40-60°C), ethyl acetate and lastly with MeOH. The petroleum ether extract, benzene extract, chloroform extract, ethyl acetate extract and methanol extract were concentrated under reduced pressure in a rotatory vacuum evaporator and stored for analysis.

RESULT AND DISCUSSION

The benzene and chloroform extract were mixed up and subjected to silica gel G column. On eluting with benzene: ethyl acetate (97.5: 2.5 v/v) collected **fractions (277-291)** which were light greenish yellow in colour. After checking purity of each fraction on TLC plate similar fractions were mixed up and washed with petroleum ether. The compound was further purified by means of TLC and HPLC methods.

IDENTIFICATION

1. Molecular formula	:	C ₁₂ H ₈ O ₄
2. Melting point	:	188°C (188-191 °C lit.)
3. Molecular weight	:	216
4. Elemental analysis	:	
(i) Found values:		C=66.59%, H=3.76%
(ii) Required values:		C=66.66%, H=3.70%

Colour Reactions :

1. It fluoresced light yellowish on silica gel G TLC plate under long range UV light (365 nm).
2. Compound was dissolved in methanol and few pellets of KOH were added to it, solution become yellow, indicating the presence of coumarin nucleus.
3. The compound gave violet colour with alkaline hydroxylamine and FeCl₃ solutions.
4. With alc. FeCl₃ compound gave dark green spot under long range UV light.
5. It formed dibromoderivative with bromine water which on treatment with alkaline gave coumarillic acid.
6. On exposure to I₂ compound gave redish brown spot.
7. Spraying the TLC with 15% H₂SO₄ it gave brown spot.
8. The compound formed addition product with sodium bisulphate.

SPECTRAL STUDIES

1. Mass spectra : M⁺ (216), 201, 188, 173, 167, 149, 145, 131, 117, 102, 89, 75, 63, 51, 201 (M – CH₃(-15), 188 (M-CO), 173 (M-CO-28).

2. UV Spectra :

λ^{MeOH} (nm) 294, 262, 248, 242, 207

Max.

The absorption bands of the compound in MeOH were characteristics of furanocoumarins. Compound did not show any change with shift reagent in NaOAc, $AlCl_3$ and H_3BO_3

3. IR Spectra: $\nu_{max}(KBr)cm^{-1}$

νcm^{-1}	Stretching
3100	due to aromatic -C-H stretching
1726	due to -C-O stretching.
1620	due to -C-C stretching.
1170	due to -C-O-C stretching.
860	due to furan ring.

IR spectra support the compound to be a **linear furanocoumarin**.

4. 1H NMR Spectra : (CDCl₃ and TMS as an internal standard.)

δppm	No of protons	δppm	No of protons
4.28	3H of OCH ₃	6.28	1H of H-3
7.02	1H of H-3'	7.27	1H of H-2'
7.58	1H of H-8	8.14	1H of H-4

5. ^{13}C NMR spectra: (CDCl₃ and TMS as an internal standard.)

Carbon atom	δppm	Carbon atom	δppm
C-2	161.2	C-3	112.5
C-8	93.8	C-9	106.3
C-4	139.2	C-10	152.6
C-5	149.5	C-2'	144.7
C-6	112.6	C-3'	105.0
C-7	158.3	OCH ₃	60.0

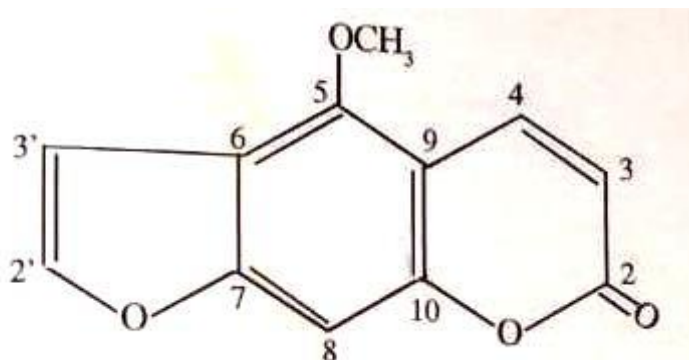
DEPT EXPERIMENT:

DEPT studies showed the presence of six quaternary carbons, five secondary carbons and one primary carbon due to methoxy group.

Colour reactions and spectral studies - **UV spectra, Mass spectra, IR spectra, ¹H NMR, ¹³C NMR spectra and DEPT** experiment concluded that the compound has a **linearfuranocoumarin** nucleus and only one methoxy group is attached at C-5 position.

On the basis of above spectral studies and literature search (Murray,R.D.H. et al., 1982, 1984 and Medina. R.J., 1976) **compound 1** was identified as **5-methoxy Psoralen or Bergapten**.

Structure



Bergapten

The compound was further confirmed by co-TLC and co-HPLC methods with an authentic sample of the compound.

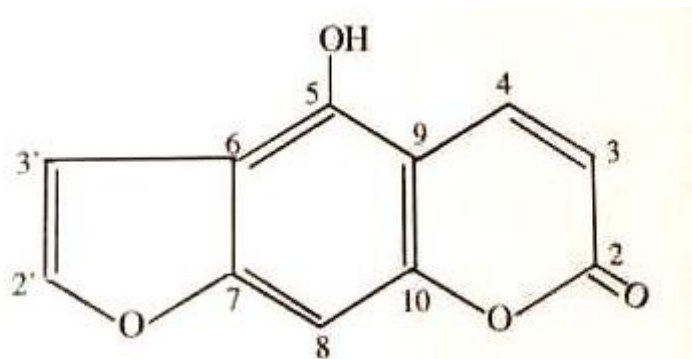
Derivative of compound 1:

Compound 1 (5 mg) was taken in round bottom flask and refluxed with 10% acetic acid and HI for 5 hrs. on cooling, the reaction mixture was extracted with solvent ether when an oily substance was obtained on evaporation of the solvent. It was recrystallised in pet. Ether and benzene as **compound 2** which gave the following results:

1. **Melting Point** : 278°C
2. **Molecular formula** : C₁₁H₆O₄
3. **Mass spectra** : m/z 202 (M⁺)

Based upon the spectral studies, MS results and comparing the values with

literature (Murray R.H.D. 1982, 1988; Conzalez, A.Z., 1977) the **compound 2** was identified as- **Bergaptol (5-hydroxy psoralen)**



Bergaptol

Compound 2

Formation of **Bergaptol** clearly indicate the position of OCH₃ group at C-5.

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