

# Preparation, Characterization and Antimicrobial and Antifungal activities of 7-Methoxy-8-[(3,3-dimethyloxiran-2-yl)methyl]-2H-chromen-2-one, an Analogue of Osthol

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## Abstract

*Osthol*, 7-methoxy-8-(3-methylbut-2-enyl) coumarin, was isolated from the root parts of *Prangos pabularia* and was subjected to modification in the isopentenyl side chain to get an epoxide 7-Methoxy-8-[(3,3-dimethyloxiran-2-yl)methyl]-2H-chromen-2-one (**1**). The structures of osthol and compound **1** were elucidated on the basis of MS, IR, <sup>1</sup>H and <sup>13</sup>C-NMR spectroscopy. Compound **1** on bio-evaluation displayed significant antimicrobial and antifungal activity.

**Key words:** *Prangos pabularia*, *Osthol*, *Epoxide*, *Antimicrobial activity*, *Antifungal activity*.

## Introduction

Genus *Prangos* (Family Apiaceae) consists of more than 36 species [1]. The plants are herbaceous, perennial, growing up to 1 m high and are mainly distributed in Mediterranean region and western and central Asia. *Prangos pabularia* is the only one species, found in India, distributed mainly in the middle Himalayan ranges, lying on the extreme north west of the sub-continent.

Osthol, 7-methoxy-8-(3-methylbut-2-enyl) coumarin, exhibits many pharmacological and biological activities [2-4]. Literature reveals that osthol has been selected for development as a hepatoprotectant [5] and as an antipruritic agent [6]. Studies with hepatitis model mice has shown that osthol has a potential of preventing hepatitis by inhibiting the development of apoptosis [7-8], indicating the possibility of osthol to become a hepatoprotective drug candidate for various liver diseases[5]. Osthol has been found to be a promising agent for the treatment of osteoporosis [9] and to demonstrate the reproductive system improvement properties by activation of the central cholinergic neuronal system [10].

## Experimental

### General

IR spectra were recorded on Perkin-Elmer Paragon-1000 spectrophotometer Esquire 3000 spectrometer. <sup>1</sup>H spectra were recorded at 400 MHz and <sup>13</sup>C NMR at 100 MHz on 500 Bruker Avanc instrument using TMS as internal standard and CDCl<sub>3</sub> as the solvent. High resolution mass spectra were recorded on Agilent (QTOF hybrid). Column chromatography was carried out on Merk silica gel (60-120 mesh and 100-200 mesh). Aluminium sheets, precoated with silica gel 60 F<sub>254</sub> (20x20 cm, 0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as spraying reagent.

### Plant Material

The root parts of *Prangos pabularia* (15 Kg) were collected from Drass, Ladakh (J&K, India) in 2013. The specimen was identified by Akhtar H. Malik, Curator, Centre for Biodiversity & Taxonomy, University of Kashmir.

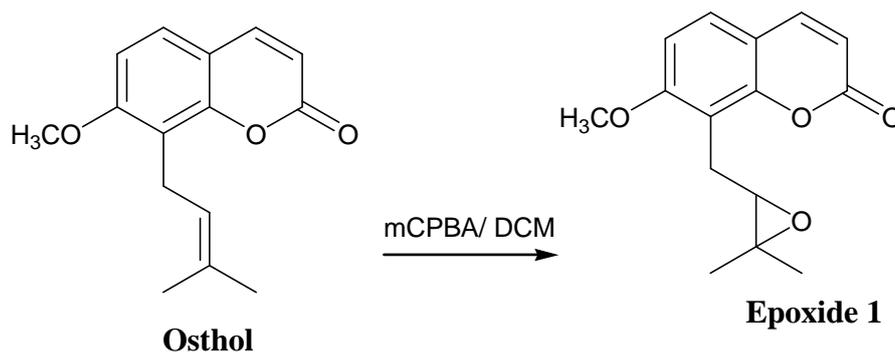
### Extraction and Isolation

The air dried, finely powdered root material (2Kg) was extracted for 72 hours sequentially with petroleum ether (60-80°C), ethyl acetate and methanol in a soxhlet apparatus to afford the respective extracts, which were concentrated under reduced pressure. Osthol was isolated from petroleum ether extract by column chromatography using silica gel as adsorbent and petroleum ether-chloroform (4:1) as eluent. Its structure was elucidated on the basis of MS, IR, UV, <sup>1</sup>H NMR and <sup>13</sup>C NMR and comparison of the spectral data with the data available in literature [11-12].

### Synthesis of 7-Methoxy-8-((3,3-dimethyloxiran-2-yl)methyl)-2H-chromen-2-one (1)

Compound **1** was prepared by dissolving osthol (1 eq.) to dichloromethane under chilled condition followed by addition of a solution of *m*-chloroperoxybenzoic acid (1.2 eq.) in dichloromethane, drop wise. The reaction completed in 2 hrs (monitored by TLC). The product was extracted with ethyl acetate and purified by silica gel column chromatography.

Epoxide (**1**) 60% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): 1.28 (6H, s, C(CH<sub>3</sub>)<sub>2</sub>), 3.0 (2H, d, *J*= 5.8 Hz, Ar-CH<sub>2</sub>), 3.19 (1H, dd, *J*= 6.03 & 8.06 Hz, Ar-CH<sub>2</sub>-CH-O-), 3.93 (3H, s, Ar-OMe), 6.25 (1H, d, *J*= 9.2 Hz, -CH=CH-CO), 6.88 (1H, d *J*=8.4 Hz, Ar-H), 7.37 (1H, d, *J*= 8.26 Hz, Ar-H), 7.65 (1H, d, *J*= 9.3 Hz, CH=CH-CO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): 19.14, 22.5, 24.76, 56.16, 59.29, 62.99, 107.43, 112.99, 113.15, 114.27, 127.7, 143.7, 153.45, 160.75, 161.01. EIMS *m/z*: 260.2741 [M<sup>+</sup>]. IR (KBr) *max*cm<sup>-1</sup> : 2924, 1731, 1607, 1384, 1280, 1252, 1111, 771, 510.



### Results and Discussion

Osthol was obtained in the form of colourless needles by extensive chromatography of the petroleum ether extract of the roots of *Prangos pabularia* over silica gel, using graded solvent systems. The compound, in its mass spectrum, showed molecular ion peak at *m/z* 244 C<sub>15</sub>H<sub>16</sub>O<sub>3</sub>. The compound gave violet colouration with alkaline hydroxylamine, followed by addition of ferric chloride, characteristic of coumarins [13]. UV-spectrum of the compound displayed absorption peaks at *max*. 317, 256, 247 nm. The IR spectrum showed prominent bands at 1727 cm<sup>-1</sup> (C=O, -unsaturated -lactone), 1601 and 1497 cm<sup>-1</sup> (aromatic), besides other bands at 1386 and 1367 cm<sup>-1</sup> (gemdimethyl).

The compound, in its <sup>1</sup>H NMR spectrum, displayed down field resonance signals due to olefinic protons at 6.18(1H, *d*, *J*=9.1Hz, H-3), 8.14(1H, *d*, *J*=9.1 Hz, H-4) and a side chain olefinic proton at 5.26(1H, *t*, H-2'). The <sup>1</sup>H NMR spectra of the compound displayed the down field resonance signals at 7.38 and 7.10 due to aromatic protons (1H, *d*, *J*=8.5 Hz, H-5) and (1H, *d*, *J*=8.5 Hz, H-6), respectively, besides a signal at 3.93, due to three singlet methoxy protons at C-7. The signals at 1.84 (3H, s), 1.81(3H, s) were assigned to C-3' gemdimethyl protons.

In the <sup>13</sup>C NMR spectrum, 15 carbon signals were observed. Nine carbons were assigned for a coumarin nucleus at *c* 160.7 (-OC=O, C-2), 112.6 (=CH, C-3), 143.4 (-CH, C-4), 117.5 (C, C-4a), 126.2 (C, C-5), 112.4 (C, C-6), 159.9 (-C-O, C-7), 107.2 (C, C-8), 152.3 (C, C-8a). The rest of the other carbon signals suggested the presence of a prenyl group at *c* 21.8 (-CH<sub>2</sub>-, C-1'), 121.0 (-CH=, C-2'), 132.3 (=C, C-3'), 25.6 (CH<sub>3</sub>, C-3'), 17.9 (CH<sub>3</sub>, C-3') and 56.1 (7-methoxy).

Comparison of physical characteristics and spectral data of the compound, with that reported in literature [12], confirmed it to be Osthol.

Compound **1**, was obtained by addition of *m*-chloroperbenzoic acid to osthol in dichloromethane under chilled conditions. Compound **21** displayed a prominent peak in IR spectrum at 771 cm<sup>-1</sup>, characteristic of trisubstituted epoxide, besides other bands at 1731 cm<sup>-1</sup> ( , -unsaturated- -lactone), 1605 & 1280 cm<sup>-1</sup>(aromatic). In proton spectrum, the chemical shift and multiplicity at C-2' supports the structure. <sup>13</sup>C NMR and mass spectrum confirmed the identity of the compound.

### Antimicrobial and Antifungal activity

Osthol and Compound **1** were bio-evaluated for their possible antimicrobial and antifungal activities (Table 1). As per the activity studies, osthol was found to be totally inactive while compound **1** showed good antibacterial activity (29 µg/ml for MRSA) as well as antifungal activity (53 µg/ml for *Candida albicans* and 51 for µg/ml *Aspergillus fumigatus*) (Table 2).

Table 1. Primary Screening: Compounds tested at 500µg/ml concentration for antibacterial and antifungal activity.

Compound	Antimicrobial		Antifungal	
	Gram +ve	Gram -ve	<i>Candida</i>	<i>Aspergillus</i>
Osthol	Inactive	Inactive	Inactive	Inactive
<b>Compound 1</b>	<b>Active</b>	<b>Active</b>	<b>Active</b>	<b>Active</b>

Table 2. Minimum inhibitory Concentration (MIC) of the Active Compound.

Compound	MIC (µg/ml)			
	<i>Staphylococcus aureus</i>	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	<i>Candida albicans</i> ATCC 90028	<i>Aspergillus fumigatus</i>
<b>1</b>	<b>230</b>	<b>29</b>	<b>53</b>	<b>51</b>

Comparison of the activity profile of osthol from *P. pabularia* and the modification product (**1**) warrant further study in the light of developing new antimicrobial, antifungal and other drugs of better therapeutic impact by chemical modifications of the parent molecule.

### Conclusion

Osthol, isolated from the root parts of *Prangos pabularia* was subjected to chemical modification and an epoxide **1** prepared. The bio-evaluation studies of compound **1** displayed good antimicrobial and antifungal activities in comparison with the parent molecule osthol.

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