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Preparation, Characterization and Antimicrobial and Antifungal activities of 2- Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al, an Analogue of Osthol, a Major Constituent from *Prangos pabularia*

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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 aldehyde 2- Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al (**1**). The structures of osthol and compound 1 were elucidated on the basis of MS, IR, ^{1}H and ^{13}C-NMR spectroscopy. Compound 1 on bio-evaluation displayed significant antimicrobial and 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. ^{1}H spectra were recorded at 400 MHz and ^{13}C NMR at 100 MHz on 500 Bruker Avanc instruement using TMS as internal standard and $CDCl_3$ 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_{254} (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 cerric 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 & Taxanomy, 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, ^{1}H NMR and ^{13}C NMR and comparison of the spectral data with the data available in literature [11-12].

Synthesis of 2-Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al (1)

Compound **1** (Scheme 1) was prepared by adding selenium dioxide (1 eq.) to a solution of osthol (1 eq.) dissolved in glacial acetic acid (7ml) and stirred for about 3 hrs. On completion of the reaction (monitored by TLC), the contents were poured into crushed ice and extracted with dichloromethane (50 ml), dried over sodium sulfate and concentrated on rotavapor to give crude product, which on silica gel column chromatography, using Pet.ether-ethyl acetate as the eluent, yielded pure aldehyde **1** in 60% yield. EIMS *m/z*: 258.2482 [M^{+}]. IR (KBr) ν_{max}cm^{-1} : 2923, 1728, 1681, 1608, 1497, 1401, 1280, 1251, 1162, 1117, 1093, 832, 774, 510. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.95 (3H, s, $\underline{CH_3}$-C=), 3.94 (3H, s, Ar-OMe), 3.89 (2H,d, *J*=7.69 Hz Ar-$\underline{CH2}$-C=), 6.28 (1H, d, *J*= 9.46 Hz, -CH=$\underline{CH}$-CO), 6.53 (1H,t, *J*= 7.34 Hz, Ar-CH_2-$\underline{CH}$=), 7.39 (1H, d, *J*= 8.62 Hz, Ar-H), 6.88 (1H, d, *J*= 8.6 Hz, Ar-H), 7.66 (1H, d, *J*= 9.5 Hz, -$\underline{CH}$=CH-CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 195.4, 160.79, 160.30, 153.0, 150.74, 143.64, 139.81, 127.38, 114.27, 113.33, 113.10, 107.4, 50.19, 22.8, 9.27.

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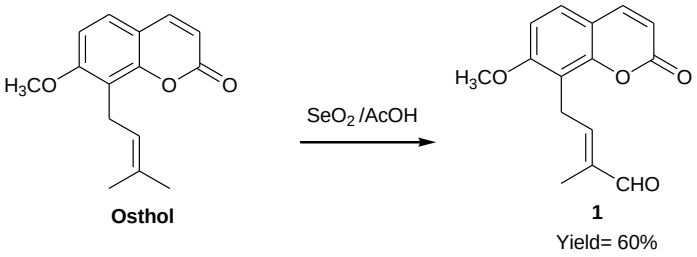

Scheme-1

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 $C_{15}H_{16}O_3$. 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 $\lambda_{max.}$ 317, 256, 247 nm. The IR spectrum showed prominent bands at 1727 cm^{-1}(α, β-unsaturated δ-lactone),1601 and 1497 cm^{-1} (aromatic), besides other bands at 1386 and 1367 cm^{-1} (gemdimethyl).

The compound, in its ^{1}H NMR spectrum, displayed down field resonance signals due to olefinic protons at δ 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 δ 5.26(1H, *t*, H-2'). The ^{1}H NMR spectra of the compound displayed the down field resonance signals at δ 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 δ 3.93, due to three singlet methoxy protons at C-7. The signals at δ 1.84 (3H, s), 1.81(3H, s) were assigned to C-3' gemdimethyl protons.

In the ^{13}C NMR spectrum, 15 carbon signals were observed. Nine carbons were assigned for a coumarin nucleus at δ_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 δ_C 21.8 (-CH_2-, C-l'), 121.0 (-CH=, C-2'), 132.3 (=C, **C**-3'**),** 25.6 (CH_3, C-3'), 17.9 (CH_3, 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**, 2-Methyl-4-(7-methoxy-2-oxo-2H-chromen-8-yl)-but-2-en-1-al, was obtained by the oxidation of osthol with selenium dioxide in acetic acid. In its IR spectrum, a prominent band at 1681 cm^{-1}, due to unsaturated aldehyde carbonyl was observed in addition to the band at 1728 cm^{-1}due to lactone carbonyl group. However, no twin peaks at 1386 & 1367 cm^{-1}, characteristic of gemdimethyl, were observed. This was further supported by proton spectrum, wherein a down field signal at δ 1.95 (singlet) for only three protons, and signal for aldehydic proton at δ 9.2, were observed. The structure was further confirmed by ^{13}C NMR and mass spectrum.

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 (32 µg/ml for MRSA) as well as antifungal activity (64 µg/ml for *Candida* and *Aspergillus*) (Table 2).

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

Compound	Antimicrobial		Antifungal	
	Gram +ve	Gram -ve	*Candida*	*Aspergillus*
Osthol	Inactive	Inactive	Inactive	Inactive
1	**Active**	**Active**	**Active**	**Active**

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

Compound	MIC (µg/ml)			
	Staphylococcus *aureus*	Methicillin-resistant Staphylococcus aureus (MRSA)	*Candida albicans* ATCC 90028	*Aspergillus fumigatus*
1	**256**	**32**	**64**	**64**

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 aldehyde **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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