

Isolation and identification of xanthotoxin (8-methoxypsoralen) from the fruits of *Heracleum persicum* Desf. ex Fischer

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Abstract

Linear furanocoumarins are toxic compounds synthesized by plants. These toxic natural products are usually found in the fruits of Umbelliferae. *Heracleum persicum* Desf. ex Fischer is an Iranian aromatic medicinal plant from Umbelliferae which their fruits are used as flavoring agent in food products. Because of the wide usage of the fruits of *H. persicum* it was decided to do a phytochemical study on the fruits of the plant. The fruits of *H. persicum* were extracted with *n*-hexane and the extract was put on an open column chromatography. Xanthotoxin (8-methoxypsoralen) was isolated from the fruits of the plant and the structure of the compound was elucidated by UV, IR, NMR and MS analyses. Due to photosensitization caused by this compound, it is recommended to use low amounts of this plant in sensitive patients. However, only further quantitative studies on total furanocoumarins of the fruits can provide definite documents for any phototoxicological hazard.

Keywords: *Heracleum persicum*; Umbelliferae; Xanthotoxin; 8-Methoxypsoralen; Furanocoumarin

INTRODUCTION

Linear furanocoumarins are toxic compounds synthesized by plants. These natural products are usually found in the fruits of Umbelliferae such as celery and parsley. One aspect of their toxicity results from the ability of UV-A photoactivated furanocoumarins to react directly and irreversibly with pyrimidine nucleotide in DNA. Another aspect of their toxicity drives from generating toxic oxyradicals. Several furanocoumarins are also used as drug for treatment of vitiligo and psoriasis (1).

The genus *Heracleum* is represented in Iran by ten species, four of them being endemic (2). *Heracleum persicum* Desf. ex Fischer (Umbelliferae) is an annual herb known as "Golpar" in Iran. The fruits of *H. persicum* are widely used as spices (3) and

the young stems are also used for making pickles. In Iranian folk medicine, the fruits of *H. persicum* were used as a carminative herbal drug (4). Because of wide usage of the fruits of *H. persicum* as medicinal plant and its use as flavouring agent, it was decided to carry out a phytochemical study on the fruits of this plant.

Chemical composition of different parts of *H. persicum* has been investigated by several authors. Pimpinellin, isopimpinellin, bergapten, isobergapten and sphonding are furanocoumarins which are reported from roots of the plant (5). There is a report that showed the presence of six furanocoumarins (6) and flavonoids (7) in the fruits of *H. persicum*.

Chemical constituents of the essential oils of fruits, leaves, stems and roots of *H. persicum* were reported previously. The fruit oil contained about 95% aliphatic

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esters, 4% aliphatic alcohols and 1% monoterpenes (8). The major components of both leaves and stems oil were reported as *trans*-anethole (9,10). Viridiflorol was also identified as predominant constituent of the volatile oil of the root of *H. persicum* (11). In this article isolation and elucidation of xanthotoxin from the fruits of this plant are described.

MATERIALS AND METHODS

General procedures

The UV spectrum was obtained using a Secomam spectrophotometer. The IR spectrum was recorded on a Perkin-Elmer 1420 instrument. The $^1\text{H-NMR}$ was recorded on a Bruker (250 MHz) instrument, using DMSO-D_6 as solvent and tetramethyl silane as internal standard. EI-MS spectrum was recorded on QP-1000EX mass spectrometer. Column chromatography was carried out on silicagel (Merck, 70-325 mesh). Compounds on the TLC (Silicagel 60GF₂₅₄ precoated plates, Merck) were detected at 365 nm by KOH as spraying reagent.

Plant material

Fruits of *H. persicum* were collected from the north of Tehran in September. The plant identity was confirmed by the Botany Department of Isfahan University. A voucher specimen of the plant was deposited in the herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran (No. 1703).

Extraction and purification

The air-dried powdered fruits (200 g) of *H. persicum* were extracted with *n*-hexane, using a soxhlet apparatus for 3 h. The solvent was removed in vacuum evaporator to obtain dried extract. The *n*-hexane fraction residue (30 g) was fractionated by column chromatography on silicagel using *n*-hexane containing solvent by increasing polarity of the mobile phase

with EtOAc (0-100%) and finally MeOH to give 10 fractions. The presence of coumarins in fractions was followed by thin layer chromatography. Samples (20 μl) of fractions were spotted on Silicagel 60GF₂₅₄ precoated plates and developed in petroleum-ether (40-60 °C): EtOAc (2.5:1). Coumarins were detected at 365 nm and KOH was used as spraying reagent.

The 6th fraction (rich of coumarins), eluted with 250 ml of *n*-hexane: EtOAc (3:7), was evaporated at reduced pressure to 50 ml and then crystallized to give white to pale yellow-colored fluffy crystals (25 mg). For further purification the crystals were rinsed with cold solvent.

Spectroscopic data

The structure of pure isolated solid crystalline (Fig. 1) was elucidated using UV, IR, Mass and $^1\text{H-NMR}$ spectra as below:

UV λ_{max} (MeOH): 210, 225, 250, 310 nm; IR (KBr): ν_{max} = 1725, 1590, 1350, 1130 cm^{-1} ; EI-MS, 70eV *m/z* (rel. int.): 216 (M^+ , 100), 201 (18), 173 (58), 145 (23), 89 (18), 69 (26), 43 (61). $^1\text{H-NMR}$ (DMSO-D_6 , δ): 3.96 (3H, s, CH_3O); 6.39 (1H, d, 3-H); 7.15 (1H, d, 4'-H); 7.29 (1H, s, 5-H); 8.01 (1H, d, 5'-H), 8.16 (1H, d, 4-H).

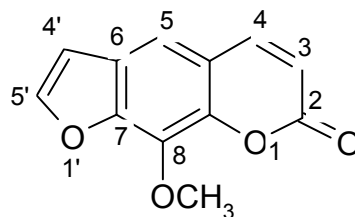


Fig. 1. Structure of xanthotoxin

RESULTS

The known furanocoumarin, xanthotoxin, was characterized by interpretation of its UV, NMR, IR and MS spectra as well as by comparison of spectral data in

literature. Coumarins exhibited a UV spectrum with four maxima in 210-350 nm (12,13), thus UV absorptions of the isolated compound ($\lambda_{\text{max}} = 210, 225, 250, 310 \text{ nm}$) indicated the presence of a coumarin nucleus.

The ^1H NMR spectrum displayed characteristic signal for a methoxy group at δ 3.96 (s). The ^1H NMR spectrum of the compound showed two proton doublets at δ 6.39 and 8.16 ($J = 10 \text{ Hz}$) characteristic for the H-3 and H-4 of the isolated compound. The presence of further two proton doublets at δ 7.15 and 8.01 ($J = 4 \text{ Hz}$) indicated the presence of H-4' and H-5' in the furan ring of coumarin respectively. The H-5 proton could be seen at δ 7.29 with a singlet form.

The EI-MS showed a molecular ion peak at m/z 216 (M^+ , base peak) in agreement with the proposed structure with $\text{C}_{12}\text{H}_8\text{O}_4$ molecular formula and ion peak at m/z 201 is due to cleavage of a methoxy moiety from the molecule. The IR spectrum with the peaks at 1725 (coumarin carbonyl), 1130 (C-O stretching) and 1350 cm^{-1} (α, β -unsaturated lactone) confirms the skeleton of xanthotoxin.

DISCUSSION

Xanthotoxin is a linear furanocoumarin occurring in many plants of the family Umbelliferae such as *Ammi majus* (14) and *Angelica japonica* (15), however, this phototoxic type of coumarin is mainly reported from different *Heracleum* species. Xanthotoxin had been previously isolated from some *Heracleum* species such as *H. mantegazzianum* (16), *H. sphondylium* (17), *H. yunnngingense* (18), *H. rapula* (19) and *H. lanatum* (20). This study indicates that the compound could be also found in the fruits of *H. persicum*.

Furanocoumarins can be exudates on the surface of the leaves of some plants (21) causing dermatitis. Xanthotoxin is one of the phototoxic furanocoumarin which

could also initialize skin dermatitis (22). Fruits of *H. persicum* are widely used as spice in Iran, thus it must be consider that the use of fruits of this plant could cause photosensitization in sensitive patients. However, only further quantitative studies on total furanocoumarins of the fruits will provide definite documents for any phototoxicological hazard.

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