ISOLATION OF FURANOCOUMARINS FROM *PASTINACA SATIVA* L. CALLUS CULTURE

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ABSTRACT

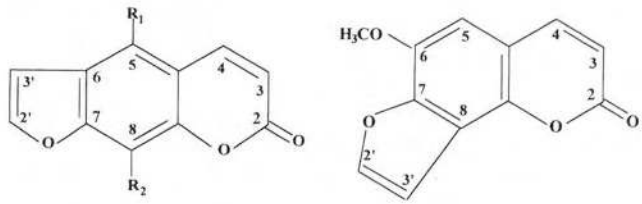
Four furanocoumarins: bergapten, xanthotoxin, isopimpinellin (linear furanocoumarins) and sphondin (angular furanocoumarin) were isolated for the first time from callus tissues of *Pastinaca sativa* L. (Apiaceae) cultured in vitro on solid medium. The compounds were identified using spectral methods. They are well-known secondary metabolites of the intact plant. This is the first report on the isolation of sphondin from in vitro plant cultures.

KEY WORDS: *Pastinaca sativa* L., Apiaceae, callus culture, linear furanocoumarins, psoralens, sphondin.

INTRODUCTION

Parsnip, *Pastinaca sativa* L., a member of the Apiaceae family, is a furanocoumarin-producing plant species. The plant reportedly contains psoralen, and its derivatives: bergapten (**1**), xanthotoxin (**2**), isopimpinellin (**3**) and imperatorin (linear furanocoumarins) as major constituents, along with sphondin (**4**), a furanocoumarin with angular structure (Fig. 1) (Beyrich 1966; Hegnauer 1973; Hoppe 1973). Psoralens, e.g. **1**-**3**, are used in therapy of skin diseases due to their photosensitising and antiproliferative activities (Pathak et al. 1981). Fruits of the plant containing high amounts of the furanocoumarins, up to ca. 1.15% (Beyrich 1966). Fruits have been recommended as an industrial source of bergapten and xanthotoxin (Głowniak 1988).

The ability of *P. sativa* callus culture to produce linear furanocoumarins was reported previously (Ekiert and Gomółka 2000). The occurrence and concentrations of the compounds were studied by HPLC using commercial coumarins as standard substances. In the course of the investigation, it became apparent that besides significant quantities of the above-mentioned linear furanocoumarins, the extract from the callus tissues contained another unidentified coumarin. The present study was aimed at the isolation of this compound, together with three major furanocoumarins detected earlier.



R_1	R_2
1 OCH_3	H
2 H	OCH_3
3 OCH_3	OCH_3

4

Fig. 1. Chemical structures of the isolated compounds: **1** (bergapten), **2** (xanthotoxin), **3** (isopimpinellin), **4** (sphondin).

MATERIAL AND METHODS

Callus culture of Pastinaca sativa L.

Hypocotyl-derived callus culture were initiated from fruits (plants grown in München-Nymphenburg Botanical Garden) as described previously (Ekiert and Gomółka 2000). The culture was maintained on a solidified Linsmaier and Skoog (1965) medium supplemented with 2 mg/dm^3 NAA and 2 mg/dm^3 BAP , under continuous light (ca. 900 lx), at 25 ±

Abbreviations:
HPLC (high-pressure liquid chromatography), TLC (thin layer chromatography), HPTLC (high-performance thin layer chromatography), NMR (nuclear magnetic resonance), EI MS (electron impact mass spectrum), BAP (6-benzylaminopurine), NAA (α-naphthaleneacetic acid).

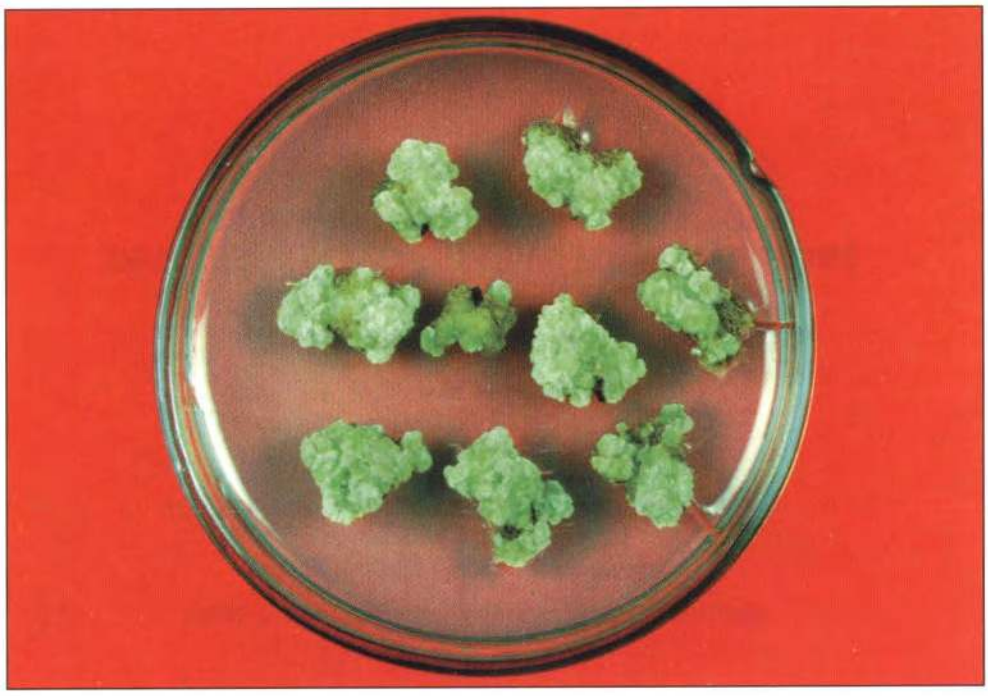


Fig. 2. Callus tissue of *Pastinaca sativa* L.

2°C (Fig. 2). The tissues were harvested every 6 weeks after inoculation in the fresh medium.

Extraction, isolation and identification of furanocoumarins

Dried, ground callus tissues (20 g) were extracted with two portions of 96% ethanol in Soxhlet's apparatus for 10 hours. The combined extracts were evaporated in vacuo to give a residue (700 mg), which was examined by TLC on silica gel (Merck, Art. 5553, n-heptane : ethyl acetate, 7 : 3, three developments, UV detection) with commercial coumarins (Serva, Roth) as standard substances, and then subjected to preparative TLC on silica gel (Merck, Art. 11844, solvent system as above, five developments). Final purification by preparative HPTLC on silica gel (Merck, Art. 5633, solvent system as above, five developments) allowed to obtain compounds: **1** (2 mg, M^+ 216) and **4** (10 mg), and two mixtures of the compounds **2** and **3** (ca 3 : 1; 7 mg and ca. 1 : 3; 15 mg, respectively), which showed two molecular ion peaks M^+ 216 and M^+ 246 in their EI mass spectra. The mixture of **2** and **3** were not separated further since ^{1}H NMR signals could be readily assigned to the respective compounds by a careful analyses of the integrals.

Sphondin (**4**). EI MS m/z (rel. int.%): 216 $[M]^+$(100.0), 201 $[M\text{-}CH_3]^+$ (35.4), 188 [M - CO]$^+$ (12.9), 173 [201 - CO]$^+$ (27.8), 145 [173 - CO]$^+$ (15.5), 89 $[C_7H_5]^+$ (6.0); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.40 (*d, J* = 9.6 Hz, H - 3), 7.75 (*d, J* = 9.6 Hz, H-4), 6.78 (*s*, H-5), 7.13 (*d, J* = 2.1 Hz, H-3'), 7.70 (*d, J* = 2.1 Hz, H-2'), 4.04 (*s*, 3H,- OCH_3).

RESULTS AND DISCUSSION

Preliminary investigations of the ethanol extract from callus tissue of *Pastinaca sativa* showed the presence of four major spots of UV-visible compounds on TLC plates, of which three corresponded to those of: bergapten (**1**), xanthotoxin (**2**) and isopimpinellin (**3**) standards on the basis of Rf value comparisons. The extract was subjected to preparative TLC and compound **4** was isolated, in addition to **1-3** (Fig. 1). The linear furanocoumarins **1-3** were identified by direct comparison of their ^{1}H NMR and EI mass spectra with those of compounds isolated previously from *Ruta graveolens* L. (Ekiert and Kisiel 1997). The identity of **4** with the angular furanocoumarin sphondin was also deduced on the spectral evidence and by comparison with reported data (Steck and Mazurek 1972).

Compounds **1-4** are well-known secondary metabolites of *P. sativa* plants growing under natural conditions (Beyrich 1966; Głowniak 1988). Previous reports on in vitro cultures of the plant derived from various plant organs (leaf, stem, root) concentrated on the culture growth conditions (Abou-Mandour 1977, 1994) and on the localisation of furanocoumarins in callus tissues (Zobel and Brown 1993).

Until now coumarins **1-3** have neither been isolated from *Pastinaca sativa* in vitro cultures, nor have they been identified by spectral methods. The compounds, however, were reported from in vitro cultures of other plant species, belonging to the Apiaceae family, e.g. from callus cultures of *Ammi majus* L. (Ekiert 1993) and *Heracleum sphondylium* L. (Tirillini and Ricci 1998), and to Rutaceae family, e.g. from *Ruta graveolens* L. cell (Steck et al. 1971) and shoot (Ekiert and Kisiel 1997) cultures. To the best of our knowledge, this paper is the first report on the isolation of the angular furanocoumarin sphondin (**4**) from plant tissues cultured in vitro.

Furanocoumarin concentrations in plants and their tissue cultures can greatly increase in result of elicitor stimulation (Tietjen et al. 1983; Hamerski and Matern 1988; Hamerski et al. 1990). It is worth mentioning that we were able to isolate compounds **1-4** from weakly differentiated callus tissues of *Pastinaca sativa* without such stimulation. Moreover, the total amount (ca. 0.4%) of linear furanocoumarins in the tissue cultures, estimated by HPLC (Ekiert and Gomółka 2000) was much higher than that in stems (ca. 0.012%), leaves (ca. 0.073%), roots (ca. 0.027%) and fruits (0.26%) of the intact plant.

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IZOLACJA FURANOKUMARYN Z KULTUR KALUSOWYCH *PASTINACA SATIV*A L.

STRESZCZENIE

Z tkanek kalusowych pasternaka zwyczajnego *Pastinaca sativa* L. (Apiaceae) hodowanych na pożywce stałej, wyizolowano po raz pierwszy i zidentyfikowano metodami spektralnymi cztery furanokumaryny: bergapten, ksantotoksynę, izopimpinelinę (furanokumaryny linearne) oraz sfondynę (furanokumaryna angularna). Związki te są znanymi metabolitami wtórnymi rośliny macierzystej. Jest to pierwsze doniesienie o izolacji sfondyny z roślinnych kultur in vitro.

SŁOWA KLUCZOWE: *Pastinaca sativa* L., Apiaceae, kultura kalusowa, furanokumaryny linearne, psoraleny, sfondyna.