COUMARINS AND ALKALOIDS IN SHOOT CULTURE OF RUTA GRAVEOLENS L.

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ABSTRACT

A shoot culture of Ruta graveolens L. (Rutacea) was maintained in the stationary liquid phase. From the cultured shoots seven compounds were isolated and identified as psoralen, bergapten, xanthotoxin, isopimpinellin (linear furanocoumarins), rutamarin (linear dihydrofuranoocumarin), kokusaginine and skimmianine (furanoquinoline alkaloids) by spectral methods. The compounds are known as secondary metabolites of the intact plant, as well as its cell and tissue cultures.

KEY WORDS: Ruta graveolens L., shoot culture, furanocoumarins, furanoquinoline alkaloids.

INTRODUCTION

Ruta graveolens L., a herb of the Rutaceae family, is a rich source of coumarins, alkaloids, flavonoids and essential oils (Hegnauer 1973). The coumarins isolated from the plant were found to possess antispasmodic (Minker et al. 1979), antibiotic (Sinha-Roy and Chakraborty 1976), photosensitizing and antiproliferating activities (Pathak et al. 1981). The antispasmodic property of the alkaloids was also described (Minker et al. 1979).

The ability of callus and cell suspension cultures of the plant to produce coumarins and alkaloids was reported previously (Petit-Paly et al., 1986). However, to the best of our knowledge, stationary liquid shoot cultures derived from this plant have not been studied. This paper deals with chemical examination of the cultured shoots.

MATERIAL AND METHODS

Stationary liquid shoot culture of Ruta graveolens L.

The culture of the plant was initiated from hypocotyl segments of sterile grown seedlings (seeds derived from Hortus Centralis Cultura Herbarum Medicarum Facultas Medica – Universitas Purkyňiana, Brno) and maintained on Petri dishes with U-shaped glass tubes covered with filter paper partly immersed into liquid Linsmaier and Skoog (1965) medium (Fig. 1), supplemented with growth substances NAA (2 mg/dm³) and BAP (2 mg/dm³), under artificial continuous illumination (900 lux), at 25 ± 2°C. The shoots were subcultured every 6 weeks.

Extraction, isolation and identification of compounds 1-7.

Dried shoots (5 g) were extracted with boiling ethanol and the solvent was removed under reduced pressure. A residue (907 mg) was directly subjected to analytical TLC on silica gel (Merck, Art. 5553, n-heptane: ethyl acetate, 7:3, three developments) with commercial coumarins (Roth, Serva) as standard compounds and then was fractionated by preparative TLC on silica gel (Merck, Art. 11844, solvent system as above, three developments). The fractionation was monitored by UV detection. Final purification of the resulting fractions on preparative HPTLC silica gel plates (Merck, Art. 5633) in the same conditions (3-5 developments) gave compounds 1 and 2 (10 mg), 3 and 4 (10 mg), 6 and 7 (5 mg) in mixtures and pure compound 5 (5 mg, M⁺ 356) (Fig. 2). The mixtures of 1 and 2 (ca 5.5 : 4.5, M⁺ 186 and M⁺ 216) 3 and 4 (ca 4 : 1, M⁺ 216 and M⁺ 246), and 6 and 7 (ca 1 : 2, M⁺ 259) were not separated further, as the 1H NMR signals could be readily assigned to the respective compounds by a careful analysis of the integrals (Table 1) and the molecular ion peaks (M⁺) in the EI mass spectra confirmed the findings.

RESULTS AND DISCUSSION

A stationary liquid shoot culture of Ruta graveolens L. was derived from hypocotyl segments of sterile grown seedlings and maintained in Linsmaier and Skoog (1965) medium supplemented with growth substances NAA (2 mg/dm³) and BAP (2 mg/dm³), under constant light (900 lux). During a subculture, the fresh biomass of shoots increased and ca. 5-fold maximum biomass increment was achieved after six weeks.
Fig. 1. Shoots of *Ruta graveolens* L. in a stationary liquid culture.

TABLE 1. $^1$H NMR spectral data of compounds 1-7 (300 MHz, CDCl$_3$, TMS as internal standard, $\delta$ – values)

<table>
<thead>
<tr>
<th>H</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>3</td>
<td>6.38 d (9.6)</td>
<td>6.27 d (9.8)</td>
<td>6.37 d (9.6)</td>
<td>6.28 d (9.8)</td>
<td>7.47 s</td>
<td>7.49 s</td>
<td>8.02 d (9.4)</td>
</tr>
<tr>
<td>4</td>
<td>7.79 d br (9.6)</td>
<td>8.15 dd (9.8, 0.5)</td>
<td>7.76 d (9.6)</td>
<td>8.12 d (9.8)</td>
<td>7.19 s</td>
<td>7.23 d (9.4)</td>
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</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>7.35 s</td>
<td></td>
<td>6.71 s</td>
<td>6.71 s</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.48 s br</td>
<td>7.14 dd (1.0, 0.5)</td>
<td></td>
<td></td>
<td>5.07 dd (7.8, 7.8)</td>
<td>7.34 s</td>
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<tr>
<td></td>
<td>2&quot;</td>
<td>7.69 d (2.3)</td>
<td>7.59 d (2.4)</td>
<td>7.68 d (2.2)</td>
<td>6.81 d (2.2)</td>
<td>7.34 s</td>
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<tr>
<td>3&quot;</td>
<td>6.83 dd (2.3, 1.0)</td>
<td>7.01 dd (2.4, 1.0)</td>
<td>6.99 d (2.3)</td>
<td>3.19 m</td>
<td>6.16 dd (17.0, 11.0)</td>
<td>3.05 d (2.8)</td>
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<tr>
<td></td>
<td>2&quot;&quot;</td>
<td>5.07 dd (17.0, 1.2)</td>
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<td></td>
<td>5.08 dd (11.0, 1.2)</td>
<td>7.04 d (2.8)</td>
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<tr>
<td>-OCH$_3$</td>
<td>4.27 s</td>
<td>4.30 s</td>
<td>4.16 s</td>
<td>4.17 s</td>
<td></td>
<td>4.02 s</td>
<td>4.03 s$^a$</td>
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<tr>
<td>-CH$_3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.03 s$^a$</td>
<td>4.11 s</td>
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<tr>
<td>Ac</td>
<td></td>
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<td>4.44 s$^b$</td>
<td>4.44 s$^b$</td>
</tr>
</tbody>
</table>

Coupling constants (Hz) in parentheses:
$^a$ 7-OCH$_3$, $^b$ 4-OCH$_3$, $^c$ 1""- methyls
The harvested shoots were dried and extracted with ethanol and the extract was examined by analytical TLC on silica gel. On the chromatographic plates four UV-visible bands were detected with the following Rf values: 0.56, 0.40, 0.35, 0.07. Of these, the Rf values 0.40 and 0.35 corresponded to those of standard coumarins: psoralen (1) and bergapten (2), and xanthotoxin (3) and isopimpinellin (4) (Fig. 2), respectively. Compounds 1-4 present in the bands of the Rf values 0.40 and 0.35, as well as compounds 5, and 6 and 7 present in the remaining bands of the Rf values 0.56 and 0.07, respectively, were separated by preparative TLC on silica gel and identified by comparison of their spectral data (1H NMR, EI MS) with those reported (Steck and Mazurek 1972).

In the plant material, the above mentioned linear furanocoumarins 1-4 were characterized, in addition to rutamarin (5) (linear dihydrofuranocoumarin) and two furanoquinoline alkaloids, kokusagine (6) and skimmianine (7). The compounds are known as secondary metabolites of the intact plant (Hoppe 1973).

Coumarins 1-3 were isolated earlier from callus of Ruta graveolens by Reinhard et al., 1968. Coumarins 1-5, and alkaloids 6 and 7 were found in cell suspension cultures of the plant by Steck et al. (1971).

Psoralens, such as 2-4 were reported previously from other plant species cultured in vitro, e.g. Petroselinum sativum L. (Reinhard 1967), Nicotiana tabacum L. (Brown and Tenniscood 1974), Annu majus L. (Ekiert 1993), Thamnosma montana Torr. & Frem. (Kutney et al. 1973). They are considered as phytoalexins in plants of the Apiaceae family (Beier et al. 1983, Beier and Oertli 1983).

In summary, the shoot culture of Ruta graveolens L. possesses ability to produce secondary metabolites and provides a convenient source for in vitro studies on elicitor—treated biosynthesis of these compounds.

LITERATURE CITED


KUMARYNY I ALKALOYDY W HODOWLI PĘDÓW RUTA GRAVEOLENS L.

STRESZCZENIE

Hodowlę pędów Ruta graveolens L. (Rutaceae) prowadzono na pożywce płynnej, w warunkach stacjonarnych. Metodami spektralnymi zidentyfikowano siedem związków wyodrębnionych z wyhodowanych in vitro pędów: psoralen, bergapten, ksantotoksynę i izopimpinelinę (linearne furanokumaryny), rutamarynę (linear- na dihydrofuranokumaryna), kokusaginę i skimianinę (alkaloidy furanochinolinowe). Związki te są znany- mi metabolitami wtórnymi rośliny macierzystej oraz jej hodowli komórkowych i tkankowych.

SŁOWA KLUCZOWE: Ruta graveolens L., hodowla pędów, furanokumaryny, alkaloidy furanochinolinowe.