COUMARINS AND ALKALOIDS IN SHOOT CULTURE OF *RUTA GRAVEOLENS* L.HALINA EKIERT, WANDA KISIEL¹⁾Chair and Department of Pharmaceutical Botany, Collegium Medicum, Jagiellonian University,
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ABSTRACT

A shoot culture of *Ruta graveolens* L. (*Rutaceae*) 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 dihydrofuranocoumarin), kokusaginine and skimmianine (furanquinoline 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 Purkyniana, 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 lx), 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 lx). 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. ¹H NMR spectral data of compounds 1-7 (300 MHz, CDCl₃, TMS as internal standard, δ – values)

H	1	2	3	4	5	6	7
3	6.38 d (9.6)	6.27 d (9.8)	6.37 d (9.6)	6.28 d (9.8)			
4	7.79 d br (9.6)	8.15 dd (9.8, 0.5)	7.76 d (9.6)	8.12 d (9.8)	7.47 s		
5			7.35 s		7.19 s	7.49 s	8.02 d (9.4)
6							7.23 d (9.4)
8	7.48 s br	7.14 dd (1.0, 0.5)			6.71 s	7.34 s	
2'	7.69 d (2.3)	7.59 d (2.4)	7.68 d (2.2)	7.62 d (2.3)	5.07 dd (7.8, 7.8)	7.57 d (2.8)	7.58 d (2.8)
3'	6.83 dd (2.3, 1.0)	7.01 dd (2.4, 1.0)	6.81 d (2.2)	6.99 d (2.3)	3.19 m. [2H]	7.05 d (2.8)	7.04 d (2.8)
2''					6.16 dd (17.0, 11.0)		
3''					5.07 dd (17.0, 1.2)		
					5.08 dd (11.0, 1.2)		
-OCH ₃		4.27 s	4.30 s	4.16 s		4.02 s	4.03 s ^a
				4.17 s		4.03 s ^a	4.11 s
						4.44 s ^b	4.44 s ^b
-CH ₃					1.50 s, 1.55 s		
					1.46 s [6H] ^c		
Ac					1.98 s		

Coupling constants (Hz) in parentheses:
^a 7-OCH₃, ^b 4-OCH₃, ^c 1''- methyls

	R ₁	R ₂
1	H	H
2	OCH ₃	H
3	H	OCH ₃
4	OCH ₃	OCH ₃

5

	R ₁	R ₂
6	OCH ₃	H
7	H	OCH ₃

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 R_f values: 0.56, 0.40, 0.35, 0.07. Of these, the R_f 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 R_f values 0.40 and 0.35, as well as compounds 5, and 6 and 7 present in the remaining bands of the R_f 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, kokusaginine (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 Tenniswood 1974), *Ammi 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. possess ability to produce secondary metabolites and provides a convenient source for in vitro studies on elicitor - treated biosynthesis of these compounds.

LITERATURE CITED

BEIER R.C., IVIE G.W., OERTLI E.H., 1983. Psoralens as phytoalexins in food plant of the family *Umbelliferae*. In: Xenobiotics in food and feeds, Finley J.W., Schwass D.E. (ed.), ACS Symp. Ser. 234, 295-310.

BEIER R.C., OERTLI E.H., 1983. Psoralen and other linear furocoumarins as phytoalexins in celery. *Phytochemistry*, 22, 2595-2597.

BROWN S.A., TENNISWOOD M., 1974. Aberrant coumarin metabolism in crown gall tumor tissue of tobacco. *Can. J. Bot.* 52: 1091-1094.

EKIERT H., 1993. *Ammi majus* L. (Bishop's weed): In vitro culture and the production of coumarin compounds. In: *Biotechnology in Agriculture and Forestry*. Vol. 21. Medicinal and Aromatic Plants IV., Bajaj Y.P.S. (ed.), Springer Verlag, Berlin - Heidelberg, 1-17.

HEGNAUER R., 1973. *Chemotaxonomie der Pflanzen*. Birkhäuser Verlag, Basel, Bd. 6, 174-230.

HOPPE A., 1973. *Drogen Kunde*. Walter de Gruyter Verlag, Berlin - New York, Bd. 2, 949-952.

KUTNEY J.P., SALISBURY P.J., VERMA A.K., 1973. Biosynthetic studies in the coumarin series. - III. Studies in tissue cultures of *Thamnosma montana*. Torr. and Frem. *The role of mevalonate*. *Tetrahedron* 29: 2673-2681.

LINSMAIER E.M., SKOOG F., 1965. Organic growth factor requirements of tobacco tissue cultures. *Physiol. Plant.* 18: 100-127.

MINKER E., BARTHA C., KOLTAI M., ROZSA Z., SCHENDREI K., REISCH J., 1979. Pharmacological study of the antispasmodic principles isolated from *Ruta graveolens*. *Planta Med.* 36: 255-256.

PATHAK M.A., PARRISH J.A., FITZPATRICK T.B., 1981. Psoralens in photochemotherapy of skin diseases. *Farmaco*, Ed. Sci. 36: 479-491.

PETIT-PALY G., RAMAWAT K.G., CHENIEUX J.C., RIDEAU M., 1986. *Ruta graveolens*: In Vitro Production of Alkaloids and Medicinal Compounds. In: *Biotechnology in Agriculture and Forestry* Vol. 7. Medicinal and Aromatic Plants II., Bajaj Y.P.S. (ed.), Springer Verlag, Berlin - Heidelberg, 488-505.

REINHARD E., CORDUAN G., VOLK O.H., 1968. Über Gewebekulturen von *Ruta graveolens*. *Planta Med.* 16: 8-16.

REINHARD E., 1967. Probleme der Produktion von Arzneistoffen durch pflanzliche Gewebekulturen. *Deutsch. Apoth. Ztg.* 107: 1201-1207.

SINHA-ROY S.P., CHAKRABORTY D.P., 1976. Psoralen, a powerful germination inhibitor. *Phytochemistry* 15, 2005-2006.

STECK W., BAILEY B.K., SHYLUK J.P., GAMBORG O.L., 1971. Coumarins and alkaloids from cell cultures of *Ruta graveolens*. *Phytochemistry* 10: 191-194.

STECK W., MAZUREK M., 1972. Identification of Natural Coumarins by NMR Spectroscopy. *Lloydia* 35: 418-439.

KUMARYNY I ALKALOIDY 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), kokusagininę i skimianinę (alkaloidy furanochinolinowe). Związki te są znanymi metabolitami wtórnymi rośliny macierzystej oraz jej hodowli komórkowych i tkankowych.

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