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COUMARINS AND ALKALOIDS FROM CELL CULTURES OF *RUTA GRAVEOLENS**

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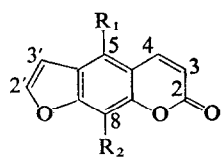
Abstract—Cells of *Ruta graveolens* L., grown in continuous light in liquid medium produced the coumarins umbelliferone, scopoletin, psoralen, xanthotoxin, isopimpinellin, rutamarin and rutacultin (6,7-dimethoxy-3-(1,1-dimethylallyl)coumarin), a new natural product. Four alkaloids were also identified: skimmianine, kokusaginine, 6-methoxydictamnine and edulinine (1-methyl-4-methoxy-3-[2,3-dihydroxy-3-methylbutyl]-2-quinolone).

INTRODUCTION

GARDEN rue (*Ruta graveolens*, Rutaceae) is an aromatic herb containing a wide variety of extractives including several furanocoumarins[1] and furanoquinolines.[2] Cell cultures of this species produce psoralen (Ia, Fig. 1) xanthotoxin (Ib) and bergapten (Ic) as well as umbelliferone (7-hydroxycoumarin), herniarin (7-methoxycoumarin) scopoletin (7-hydroxy-6-methoxycoumarin) and rutaretin (2′-hydroxyisopropyl-8-hydroxy-2′,3′-dihydropsoralen).[3] We have grown liquid suspension cultures of this species and have found a somewhat different series of coumarin derivatives, including one novel coumarin, along with a series of furanoquinoline alkaloids and the quinolone alkaloid edulinine.

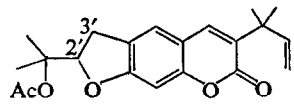


Ia PSORALEN $R_1 = R_2 = H$.

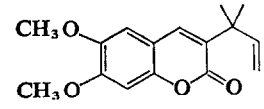
Ib BERGAPTEN $R_1 = OCH_3$, $R_2 = H$.

Ic XANTHOTOXIN $R_1 = H$, $R_2 = OCH_3$

Id ISOPIMPINELLIN $R_1 = R_2 = OCH_3$.



II RUTAMARIN.



III RUTACULTIN.

FIG. 1.

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[2] G. SCHNEIDER, *Planta Med.* **13**, 425 (1965).
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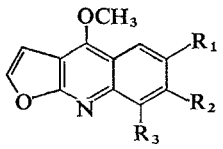

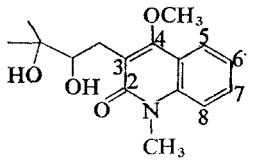

V RUTANINE

IVa SKIMMIANINE $R_1 = H; R_2 = R_3 = OCH_3$.

IVb KOKUSAGININE $R_1 = R_2 = OCH_3; R_3 = H$.

IVc 6-METHOXYDICTAMNINE $R_1 = OCH_3; R_2 = R_3 = H$.

FIG. 1. COMPOUNDS ISOLATED FROM SUSPENSION CULTURES OF *Ruta graveolens*.

RESULTS AND DISCUSSION

Umbelliferone, scopoletin, psoralen, xanthotoxin and bergapten were found in the cells, as previously reported.[3] Herniarin and rutaretin were not detected, but no intensive search for the latter was carried out. Three other coumarin derivatives were found. The first, isopimpinellin (Id), has not been reported from this species and could not be detected by GLC in the coumarin fraction from 2·4 kg fresh plant shoots from the greenhouse (harvested prior to flowering); in the cultures, it was present in large amounts (Table 1). The second,

TABLE 1. CONCENTRATION OF COUMARINS IN *R. graveolens* CULTURES

Compound	Concentration	
	mg/kg fr. wt.	g/kg dry wt.
Psoralen	5–10	0·1–0·2
Xanthotoxin	30–40	0·6–0·8
Bergapten	100–120	2·0–2·4
Isopimpinellin	60–80	1·2–1·6
Rutamarin	5–10	0·1–0·2
Rutacultin	20–30	0·4–0·6

rutamarin[4] (II), was present only in traces in plant shoots. Its structure was confirmed by the identity of its u.v. and NMR spectra with those of heliettin acetate prepared from authentic heliettin.[5] The third, m.p. 100–102° from hexane, was not detectable in plant shoot extracts. It fluoresced bright blue in u.v. light, exhibited no phenolic group, and possessed u.v. absorption maxima at 235, 253, 260, 277, 294 and 341 nm, indicating a 6,7-dialkoxycoumarin structure. The NMR spectrum revealed two aromatic methoxyls which must reside at C-6 and C-7. The presence of two aromatic singlet protons further confirmed this. The remainder of the spectrum was very similar to that of rutamarin, indicating a 1,1-dimethylallyl group at C-3. This new natural product was accordingly assigned structure III. The correctness of

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this formulation was later confirmed by comparison of the isolated substance with synthetic material kindly provided by Prof. R. D. H. Murray. We propose the trivial name *rutacultin* for this coumarin.

Of the furanoquinoline alkaloids, kokusaginine (IVb) was most abundant in the cultures whereas skimmianine (IVa) was the predominant alkaloid in greenhouse plants. Both cultured cells and whole plants produced in addition small amounts of a third furanoquinoline derivative which was tentatively identified as 6-methoxydictamnine (IVc) by its GLC behaviour, its u.v. spectrum and by its NMR spectrum which, due to the scarcity of sample, showed clearly only methoxyl protons at δ 3·95 and 4·46.

The other alkaloid, m.p. 138–141° from benzene: hexane, could not be detected in greenhouse plants. Its u.v. spectrum resembled that of 4-methoxy-1-methyl-2-quinolone. The i.r. spectrum [KBr] disclosed the presence of hydroxyl groups, an *ortho*- disubstituted benzene ring (748 cm^{-1}) and a chelated carbonyl function (1625 cm^{-1}). The NMR spectrum gave signals identical with those found for edulinine (V) isolated from other rutifers[6,7] and, as the other spectra were in agreement, we concluded our compound was edulinine. This compound has not been found previously in this genus.

EXPERIMENTAL

The cultures, obtained from Prof. E. Reinhard through Dr. S. A. Brown, were grown in continuous light in liquid B5 medium containing casein hydrolyzate.[8,9] Several batches were examined by the following procedure.

About 1000 g fresh cells (about 50 g dry wt.) were filtered from the medium and washed with water. These were then homogenized in 4 l. boiling methanol and suction-filtered. The filtrate was diluted with 1 l. water and shaken once with hexane to remove chlorophylls, lipids and oils. The methanol was removed from the separated aqueous layer; the residual suspension (2 l.) was treated with 50 ml conc. HCl and extracted twice with $CHCl_3$ to remove a coumarin-rich fraction. 50 g solid NaOH was dissolved in the remaining aqueous solutions, which was then re-extracted with $CHCl_3$ to yield an alkaloid fraction. The coumarins were fractionated on silicic acid as described elsewhere.[10] The alkaloids were examined by GLC under reported conditions[11] except that a 1·5 m column was used.

The identities of the previously reported compounds psoralen, xanthotoxin and bergapten were demonstrated by m.p., u.v. spectrum, GLC behaviour and NMR spectra (TMS internal standard). Authentic standards were not available for 6-methoxydictamnine or edulinine.

Isopimpinellin. M.p. 148–150° (lit. 148–151°).[12] Mixed m.p. undepressed. U.v. λ_{max} 224, 241, 248, 269 and 312 nm. NMR [$DCCl_3$]: Doublets at δ 8·08 and 6·24 (both J = 9·5 c/s, H-3 and H-4); doublets at 7·59 and 6·96 (both J = 2·5 c/s, H-2′ and H-3′); singlet at 4·14 (6H of aromatic methoxyls).

Rutamarin. M.p. 104–6° (lit. 107–8°).[4] U.v. λ_{max} 227, 248, 258, 297 and 332 nm. NMR [$DCCl_3$]: Singlets at δ 7·48(H-4), 7·19 (H-5) and 6·71 (H-8); 6·18 (quartet, dimethylallyl H-2); 5·1 (triplet, J = 8, H-2′); 5·07 and 5·04 (doublets of doublets, terminal CH_2); 3·19 (doublet, J = 8, 2H-3′); 1·99 (singlet, acetyl 3H); 1·56 and 1·51 (singlets, methyls of 2′-isopropyl group); and 1·49 (singlet, methyls of dimethylallyl group).

Rutacultin. M.p. 100–2° (Auth: 102–3°). U.v. λ_{max} 235, 253, 260, 287, 294 and 341 nm. NMR [$DCCl_3$]: Singlets at δ 7·49 (H-4), 6·85 (H-5) and 6·81 (H-8); 6·20 (quartet, dimethylallyl H-2); 5·06 and 5·04 (doublets of doublets, terminal CH_2); 3·92 and 3·89 (singlets, aromatic methoxyls); and 1·50 (singlet, methyls of dimethylallyl group).

Kokusaginine. M.p. 165–168° (lit. 168–9°).[13] Mixed m.p. undepressed. U.v. λ_{max} 244, 251, 296, 308, 320 and 334 nm. NMR [$DCCl_3$]: Doublets at δ 7·57 and 7·04 (both J = 2·5, H-2′ and H-3′); singlets at 7·49 and 7·36 (H-5 and H-8); singlets at 4·44 (4-methoxyl) and 4·03 (6- and 7-methoxyls). See Ref. 14.

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Skimmianine. M.p. 173–176° (lit 176·5°).[13] Mixed m.p. undepressed. U.v. λ_{max} 250, 319 and 332 nm. NMR [$DCCl_3$]; Doublets at δ 7·57 and 7·02 (both $J = 2·5$, H-2′ and H-3′); doublets at 8·01 and 7·23 (both $J = 9·5$, H-5 and H-6); singlets at 4·42 (4-methoxyl), 4·12 (8-methoxyl) and 4·02 (7-methoxyl). See Ref. 14.

6(?)-*Methoxydictamnine.* U.v. λ_{max} 245, 283sh, 294, 305, 332 and 348 nm. See Ref. 15. NMR [$DDCl_3$]: Doublet at δ 7·95 ($J = 9$–10 c/s); possible doublet (also $J = 9$–10 c/s) at 7·03: doublet at 7·64 ($J = 2$–3 c/s, furan ring proton); strong singlets at 4·46 (4-methoxy) and 3·95 (6?-methoxy). Other signals obscured.

Edulinine. M.p. 138–141° (lit. 140–1°).[7] U.v. λ_{max} 233, 273, 282, 323 and 337 nm. See Ref. 7. NMR [$DCCl_3$]: Four adjacent aromatic protons (δ 7·3–7·85); singlet at 3·97 (4-OCH_3); singlet at 3·75 (N-CH_3); multiplet at 3·59 (CH) and doublets of doublets at 3·11 and 2·79 (CH_2) coupled to the 3·59 proton in an AMX system ($J_{AM} = 14$ c.p.s., $J_{AX} = 2$, $J_{MX} = 10$); singlet at 1·34 (gem-dimethyls); broad singlets at 2·60 (OH) and 5·01 (chelated OH).

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