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THE ROLE OF 5,8-DIHYDROXYPSORALEN IN THE BIOSYNTHESIS OF ISOPIMPINELLIN

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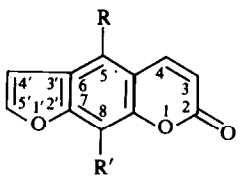
Key Word Index—*Ruta graveolens*; Rutaceae; biogenesis; *O*-alkylfuranocoumarins; 5,8-dihydroxypsoralen; isopimpinellin (5,8-dimethoxypsoralen).

Abstract—[^{3}H]5,8-Dihydroxypsoralen was effectively and selectively converted into 5,8-dimethoxypsoralen by roots of *Ruta graveolens* whereas [^{3}H]8-methoxypsoralen and [^{3}H]5-methoxypsoralen included for comparison were converted to a much lesser extent. These results indicate that 5,8-dihydroxypsoralen is a major physiological precursor of isopimpinellin.

INTRODUCTION

Psoralens (linear furanocoumarins) are a well-known group of natural and synthetic compounds [1] which act as photosensitizers of various biological substrates [2, 3]. Two natural furanocoumarins, 8-methoxypsoralen (**3**) (8-MOP, xanthotoxin) and 5-methoxypsoralen (**2**) (5-MOP, bergapten) are widely used in the photochemotherapy of vitiligo and psoriasis [4–7].



1 R = R′ = −H
2 R = −OMe, R′ = −H
3 R = −H, R′ = −OMe
4 R = R′ = −OMe
5 R = R′ = −OH

The picture concerning the biogenesis of this class of substances is fairly complete [1], even though some aspects do warrant further study. One of these is the biosynthesis of the 5,8-di-*O*-alkylpsoralens. Brown and Sampathkumar [8], working with *Heracleum lanatum* and cell cultures of *Ruta graveolens*, showed that formation of 5,8-dimethoxypsoralen (isopimpinellin) (**4**) from xanthotoxin (**3**) and from bergapten (**2**) is possible via C-5 or C-8 hydroxylation and successive *O*-methylation. Other studies, however, have shown that psoralen (**1**) can act as a precursor of isopimpinellin (**4**) [9], with an efficiency only a little less than that shown by xanthotoxin (**3**) [8]. It was of interest to investigate whether a third pathway other than the two mentioned above could exist, involving the conversion of psoralen (**1**) to isopimpinellin (**4**) via 5,8-dihydroxypsoralen (**5**).

In this study, the possible operation of a third pathway has been investigated by feeding [^{3}H]5,8-dihydroxypsoralen (**5**) and, for comparison, labeled xanthotoxin (**3**) and bergapten (**2**), to the roots of *R. graveolens* and then measuring their conversion into isopimpinellin (**4**), bergapten (**2**) and xanthotoxin (**3**).

RESULTS AND DISCUSSION

[^{3}H]5,8-Dihydroxypsoralen (**5**) was prepared from [^{3}H]xanthotoxin (**3**) by oxidation to 5,8-psoralenquinone [10] followed by reduction [11]. The ^{3}H-labelled substrate, dissolved in water and stabilized with a small amount of sulphur dioxide, was immediately fed to the roots of *Ruta graveolens*. By using this procedure the oxidative changes of 5,8-dihydroxypsoralen which occur in aqueous solution [8] were reduced, at least for the time necessary for the absorption of the solution by the root. This part of the plant was chosen as isopimpinellin was present in isolatable amounts [12]. After the incubation period, the roots were dried, triturated and extracted with methanol and the extract worked-up to obtain the 'coumarinic extract' [13–15]. In analogous experiments, labeled bergapten (**2**) and xanthotoxin (**3**) were administered to the plant.

Bergapten (**2**), xanthotoxin (**3**) and isopimpinellin (**4**) were isolated from the 'coumarinic extract' by TLC [16]. The furanocoumarins were eluted with ethanol and the solutions obtained used for spectrophotometric determinations and for radioassay [17].

The results (Table 1) show that there was a very effective conversion of 5,8-dihydroxypsoralen into isopimpinellin. On the other hand, the incorporation of this precursor into xanthotoxin was markedly lower, and much lower, into bergapten. The limited incorporation of the precursor into xanthotoxin and bergapten, when compared to that incorporated into isopimpinellin, could be explained by assuming a requirement for a dehydroxylation reaction, as suggested by Brown and Sampathkumar [8].

In parallel experiments carried out using labeled xanthotoxin, this furanocoumarin was converted into 5,8-dimethoxypsoralen, but to an extent 17 times less than

Table 1. Incorporation of radioactivity from [^{3}H]5,8-dihydroxypsoralen (8.22×10^6 dpm/μmol) into isopimpinellin, bergapten and xanthotoxin, in *Ruta graveolens* roots

Period of incubation (hr)	Isopimpinellin		Bergapten		Xanthotoxin	
	(10^{-3} dpm/μmol)	D*	(10^{-3} dpm/μmol)	D*	(10^{-3} dpm/μmol)	D*
96	437.38	19	0.83	9,800	22.64	363
96	473.01	17	0.69	11,903	21.39	384
96	455.59	18	1.29	6,362	37.02	222

*Dilution: ratio of the sp. act. of the administered compound and of the isolated furanocoumarin.

Table 2. Incorporation of radioactivity from [^{3}H]5-methoxypsoralen (6.97×10^6 dpm/μmol) and from [^{3}H]8-methoxypsoralen (14.97×10^6 dpm/μmol) into isopimpinellin, xanthotoxin and bergapten

Administered compound	Period of incubation (hr)	Isopimpinellin		Xanthotoxin		Bergapten	
		(10^{-3} dpm/μmol)	D*	(10^{-3} dpm/μmol)	D*	(10^{-3} dpm/μmol)	D*
Bergapten	96	2.50	2,786	1.77	3,938	151.15	46
	96	3.10	2,247	1.45	4,820	170.24	41
Xanthotoxin	96	46.41	322	400.91	37	1.50	9,890
	96	50.43	297	416.45	36	1.77	8,445

*Dilution: ratio of the sp. act. of the administered compound and of the isolated furanocoumarin.

that observed with 5,8-dihydroxypsoralen. Finally, the efficacy of conversion of bergapten into isopimpinellin was *ca* 170 times lower than that observed with 5,8-dihydroxypsoralen (see Table 2). This finding is in line with the previous findings of Brown, who reported that xanthotoxin could be converted more effectively than bergapten into isopimpinellin in the leaves of *Heracleum lanatum* and in *Ruta* cells [8].

The very effective and selective conversion of 5,8-dihydroxypsoralen into isopimpinellin, with low dilution values, in comparison with xanthotoxin and bergapten, suggests that this compound is on the major biosynthetic pathway to 5,8-dimethoxypsoralen in *Ruta*. Final proof of such a role might be obtained by trapping experiments. However, in a test carried out by administering labeled marmesin together with unlabeled 5,8-dihydroxypsoralen to the roots of *R. graveolens*, it was not possible to observe the presence, in the coumarinic extract, of 5,8-dihydroxypsoralen. This question, therefore, remains unresolved.

EXPERIMENTAL

Feeding procedure. Freshly gathered roots of *Ruta graveolens* were fed in 100 ml beakers with an aq. soln (25 ml) of [^{3}H]**5** (2 mg). The system was illuminated with 500 W Osram HWL lamps and the levels of the liquid layers were kept constant by addition of nutrient soln [18]. The incubation period lasted 96 hr.

In parallel expts the roots of *Ruta* were fed with aq. solns (25 ml) of either [^{3}H]**3** (2 mg) or [^{3}H]**2** (2 mg).

Radiochemical purity of isolated furanocoumarins. The radiochemical purities of **2**–**4** were confirmed by TLC (Si gel; EtOAc–cyclohexane, 1:2). Most of the radioactivity applied to the plate migrated with **2**–**4** and the sp. act. of the three furanocoumarins recovered from the plates was unaltered on further TLC.

Determination of specific radioactivity. The sample dissolved in EtOH was assayed spectrophotometrically. An aliquot of the EtOH soln was then added to 10 ml of a dioxane-based liquid scintillator (containing g/l. of dioxane: naphthalene 120; PPO 4; POPOP 0.075) and the radioactivity measured in a liquid scintillation spectrometer. The counting efficiency for ^{3}H was in the range 32/37%.

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