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BIOGENESIS OF LINEAR *O*-ALKYLFURANOCOUMARINS: A NEW PATHWAY INVOLVING 5-HYDROXYMARMESIN

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Key Word Index—*Ficus carica*; Moraceae; *Ruta graveolens*; Rutaceae; biogenesis; *O*-alkylfuranocoumarins; marmesin; 5-hydroxymarmesin.

Abstract—Radioactivity from [^{3}H] 5-hydroxymarmesin was incorporated into 5-methoxypsoralen by administration to leaves of *Ficus carica* and cut ends of *Ruta graveolens*. No other furanocoumarins were labelled. Trapping experiments, in which [^{3}H]marmesin together with 5-hydroxymarmesin was administered to fig leaves and to cut ends of rue, provided good evidence that 5-hydroxymarmesin is formed by hydroxylation of marmesin. These results, together with those obtained previously with 8-hydroxymarmesin demonstrate that, in addition to the pathway which involves the hydroxylation of psoralen, the *O*-alkylfuranocoumarins are also formed by a pathway which involves the hydroxylation of marmesin.

INTRODUCTION

Linear furanocoumarins, or psoralens, are a group of naturally occurring compounds which have marked photosensitizing effects on human skin and on various other biological materials [1,2]. Thus one form of dermatitis in man is caused by exposure to sunlight of skin which has been in contact with a plant containing furanocoumarins. Two natural, linear *O*-alkylfuranocoumarins, 8-methoxypsoralen (**8**) (8-MOP) (xanthotoxin) and 5-methoxypsoralen (**10**) (5-MOP) (bergapten) are widely used in the photochemotherapy of psoriasis and of other skin diseases [3–6].

The biogenesis of linear furanocoumarins has been largely elucidated (Fig. 1), apart from the later stages in the biogenesis of the linear *O*-alkylfuranocoumarins. In this connection, two pathways have been suggested. The first, proposed by Brown *et al.* [7] and supported by our own experimental findings [8, 9], involves the hydroxylation of psoralen (**6**) at C-5 or C-8 followed by *O*-methylation of the newly introduced hydroxyl group. The second pathway, proposed by our group [10], involves the hydroxylation of the 4′,5′-dihydrofuranocoumarin precursor of psoralen (marmesin, **5**) at C-5 or C-8 followed by aromatization of the furan ring and *O*-alkylation of the hydroxyl group. We have suggested that this second pathway may be operative in plants in which psoralen (**6**) is accumulated [10] and that the first pathway is operative in those plants in which psoralen is not accumulated. Experimental evidence for the hydroxylation of marmesin (**5**) at C-8 in the biosynthesis of 8-*O*-alkylfuranocoumarins has been obtained. Thus rutaretin (**7**), the 8-hydroxy derivative of marmesin, is present in *Ruta graveolens* and is the specific physiological precursor of 8-methoxypsoralen (**8**) in this plant [10].

In this paper, we present experimental evidence to show that 5-methoxypsoralen can be formed from marmesin via 5-hydroxymarmesin. Some of the results reported have already been published as a short communication [11].

RESULTS AND DISCUSSION

Synthesis of 5-hydroxymarmesin

To carry out the experiments designed to provide further experimental support for the second route to 5-methoxypsoralen, it was necessary to prepare 5-hydroxymarmesin (**9**), a compound that had neither been isolated from plants† nor prepared by synthesis. (±)-5-Hydroxycolumbrianetin was prepared by rearrangement and cyclization from 5-hydroxyprenyl-7-hydroxycoumarin. The α-pyrone ring of this compound was opened by treatment with alkali and then recyclized in acidic solution to form (±)-5-hydroxycolumbianetin and (±)-5-hydroxymarmesin [12]. The (±)-5-hydroxymarmesin was separated from the (±)-5-hydroxycolumbianetin but because of the low yield no attempt was made to separate the two enantiomers.

Conversion of 5-hydroxymarmesin (**9**) *into 5-methoxypsoralen* (**10**)

An aqueous solution of [^{3}H]-(±)-5-hydroxymarmesin was administered to leaves of *Ficus carica* and to cut ends of *R. graveolens*. At the end of the incubation periods (Table 1) coumarinic extracts were prepared and worked up for ^{3}H-labelled 5-methoxypsoralen (**10**), psoralen (**6**) and 8-methoxypsoralen (**8**) (present only in *R. graveolens*) (Table 1). The results show that 5-hydroxymarmesin was converted into 5-methoxypsoralen both in fig leaves and rue, but that little was converted either into psoralen or 8-methoxypsoralen, cf. incorporation of 8-hydroxymarmesin (**7**) (rutaretin) into 8-methoxypsoralen in *R. graveolens* [10].

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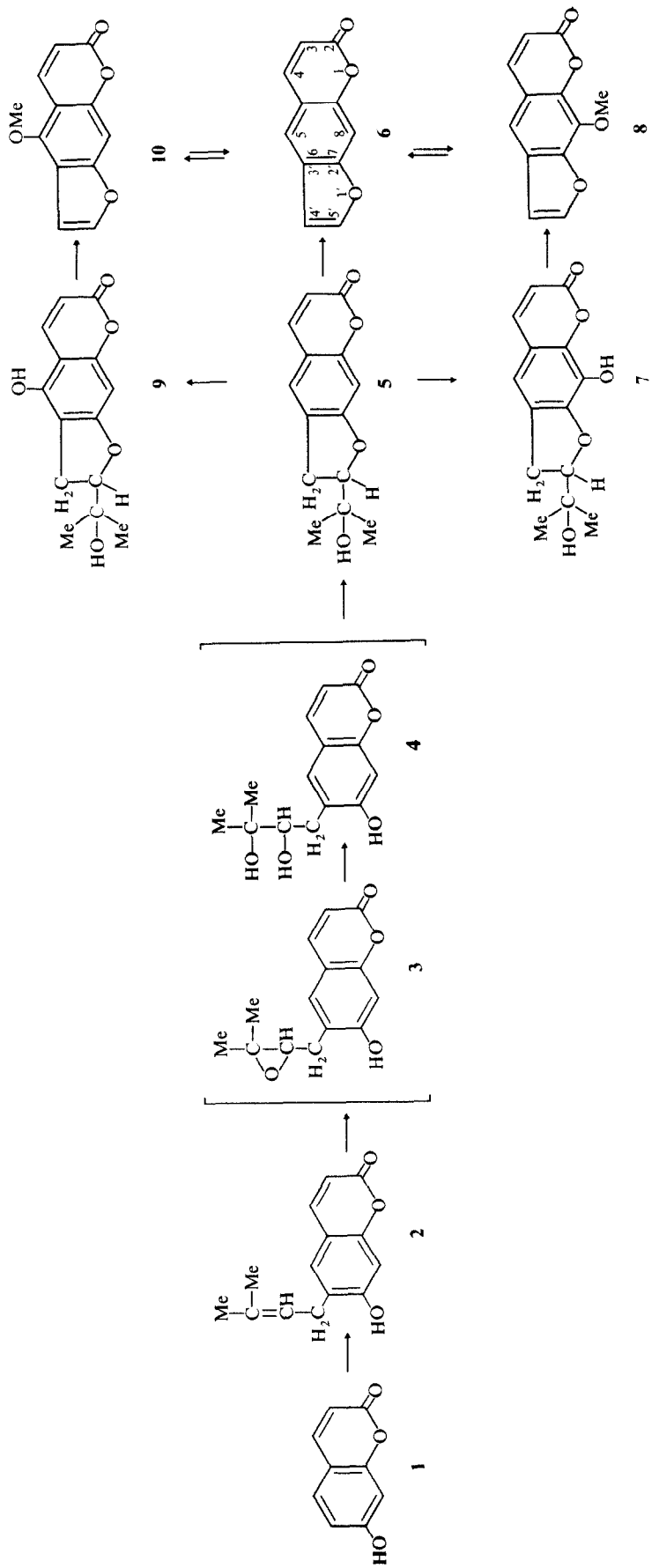
† Recently the glucoside of 5-hydroxymarmesin has been isolated from *Apium graveolens*; Garg, S. K., Gupta, S. R. and Sharma, N. O. (1980) *Planta Med.* **38**, 363.



Fig' 1. Proposed pathways for the biosynthesis of *O*-alkylfuranocoumarins.

Table 1. Incorporation of radioactivity from (±)-[^{3}H]-5-hydroxymarmesin (6.53×10^7 dpm/μmol) into psoralen, 5-methoxypsoralen and 8-methoxypsoralen

Plant species	Period of Incubation (hr)	Psoralen		5-Methoxypsoralen		8-Methoxypsoralen	
		(10^{-5} × dpm/μmol)	D*	(10^{-5} × dpm/μmol)	D*	(10^{-5} × dpm/μmol)	D*
F. carica	72	—	—	1.53	425	Not present	
	72	0.004	167 000	2.45	266	Not present	
	72	—	—	0.76	854	Not present	
R. graveolens	96	—	—	0.69	945	—	—
	96	0.006	118 000	3.04	215	0.004	168 000
	96	—	—	0.37	1754	—	—

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

Biosynthesis of 5-hydroxymarmesin (**9**) *from marmesin* (**5**)

An aqueous solution of [^{3}H] marmesin and unlabelled (±)-5-hydroxymarmesin was administered to fig leaves and cut ends of rue for the same periods of time that were used in previous trapping experiments [10, 13]. At the end of the incubation periods, the leaves and plants were dried, triturated and extracted with ether. The 5-hydroxymarmesin was partitioned into aqueous $NaHCO_3$ solution and then, after acidification, partitioned into ether. The marmesin, the furanocoumarins in the original ether extracts and the 5-hydroxymarmesin were purified by TLC. The compounds were not isolated via the preparation of the classic 'coumarinic extract', since the first step in the preparation of such an extract (treatment of a concentrated methanolic extract with NaOH at pH values > 12) can bring about a molecular rearrangement of 5-hydroxymarmesin to 5-hydroxycolumbianetin [12]. The results of these experiments (Table 2) show that the 5-hydroxymarmesin isolated from the plant tissues trapped a significant amount of the administered radioactivity, as shown by the specific radioactivity and dilution values. These findings provided further support for the proposal that 5-hydroxymarmesin (**9**) is formed by hydroxylation of marmesin (**5**). The results also showed, as demonstrated previously [7, 14], that marmesin is converted into psoralen more effectively than into *O*-alkylfuranocoumarins. However, the pathway from marmesin to psoralen is shorter than from marmesin to 5-methoxypsoralen and 8-methoxypsoralen. The extent of the conversion of marmesin into 5-methoxypsoralen was the same order of magnitude as the conversion of marmesin into 5-hydroxymarmesin. When allowance is made for the fact that further steps are involved in the transformation of 5-hydroxymarmesin to 5-methoxypsoralen, these data seem consistent with the biosynthesis of 5-methoxypsoralen both via 5-hydroxymarmesin and via 5-hydroxypsoralen.

Conclusions

The results of the experiments reported above strongly support the biosynthesis of 5-hydroxymarmesin (**9**) from marmesin (**5**). Moreover, the effective conversion of 5-hydroxymarmesin into 5-methoxypsoralen, both in leaves of *F. carica* and in cut ends of *R. graveolens*, supports the existence of a new biogenetic pathway to bergapten via hydroxylation of marmesin at C-5. From a consideration of these new results and those previously obtained with 8-hydroxymarmesin (**7**) (rutaretin) [10], we suggest that the hydroxylation of marmesin at C-5 or C-8 should be considered as the first biochemical step of a general biogenetic pathway for the formation of linear 5- and 8-*O*-alkylfuranocoumarins which is complementary to the one involving hydroxylation of psoralen.

EXPERIMENTAL

Preparation of ^{3}H*-labelled* (±)*-5-hydroxymarmesin* (**9**) *and marmesin* (**5**). (±)-**9**, prepared by synthesis in this institute [12], and **5**, extracted from *Aegle marmelos* Correa [15], were tritiated at the Radiochemical Centre (Amersham, U.K.) by a catalytic exchange method (TR 8). The compounds were purified by a procedure described previously [16]. The sp. activities of the samples used in the expts are given in Tables 1 and 2.

Experiments with [^{3}H]-**9**. Freshly cut leaves of *F. carica* and cut ends of *R. graveolens* (fig leaves in test-tubes and cut ends of rue in 100 ml beakers) were each fed with an aq. soln (12 ml) of [^{3}H]-**9** (2 mg). The systems were illuminated with 500 W Osram HWL lamps and the levels of the liquid layers were kept constant by additions of nutrient soln [17]. The incubation periods are given in Table 1.

Trapping experiments. The conditions used were the same as those just described, except that the aq. soln of [^{3}H]-**9** was replaced with an aq. soln (16 ml) of [^{3}H]-**5** (2 mg) and **5** (8 mg). The incubation periods are given in Table 2.

Isolation and purification of furanocoumarins, **5** *and* **9**. Psoralen (**6**), 5-methoxypsoralen (**10**) and 8-methoxypsoralen (**8**) were isolated by a procedure described elsewhere [13, 16, 18–20]. To obtain **5** and **9** the vegetable material was extracted with Et_2O and the extract washed (× 3) with an equal vol. of satd. $NaHCO_3$. Compound **5** and the furanocoumarins in the Et_2O phase were purified by the procedure referred to above [13, 16, 18–20]. The aq. $NaHCO_3$ phase was acidified with 2 M HCl and then extracted with Et_2O. After evapn of the Et_2O, **9** was isolated from the residue by prep. TLC on Si gel developed with $CHCl_3$–MeOH (9:1, v/v) (R_f 0.35), followed by TLC on Si gel developed with EtOAc–cyclohexane (3:1, v/v) (R_f 0.31). The radiochemical purity of **9** was confirmed by TLC on Si gel developed with $CHCl_3$–MeOH (22:3, v/v). Most of the radioactivity applied to the plate migrated with **9** and the sp. activity of **9** recovered from the plate was unaltered on further TLC. The sp. activities of **5**, **6**, **8** and **10** also remained constant after further TLC.

Determination of sp. radioactivity. The sample was dissolved in EtOH and 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,

Table 2. Trapping experiments using [^{3}H]marmesin (83×10^5 dpm/μmol) and (±)-5-hydroxymarmesin

Plant species	Period of incubation (hr)	Marmesin (10^{-5} × dpm /μmol)	Marmesin D*	5-Hydroxymarmesin (10^{-5} × dpm /μmol)	5-Hydroxymarmesin D*	Psoralen (10^{-5} × dpm /μmol)	Psoralen D*	5-Methoxypsoralen (10^{-5} × dpm /μmol)	5-Methoxypsoralen D*	8-Methoxypsoralen (10^{-5} × dpm /μmol)	8-Methoxypsoralen D*
R. graveolens	48	19.7	4.2	0.072	1148	0.138	600	0.074	1289	0.094	878
	48	15.6	5.3	0.127	654	0.208	399	0.138	600	0.091	916
	48	—	—	0.045	1838	—	—	—	—	—	—
	48	29.7	1.0	0.026	1080	0.136	208	0.047	599	0.031	919
F. carica	36	48.3	1.7	0.098	847	0.028	2939	0.031	2654	Not present	
	36	48.6	1.7	0.087	948	0.179	461	0.161	516	Not present	
	36	—	—	0.108	766	—	—	—	—	Not present	
	36	27.9	1.0	0.051	559	0.193	146	0.038	736	Not present	

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

4; POPOP, 0.075) and the radioactivity measured in a liquid scintillation spectrometer. The counting efficiency for ^3H was in the range 32–37%.

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