The Role of 5-Hydroxymarmesin in the Biogenesis of Bergapten

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5-hydroxymarmesin administered to leaves of *Ficus carica* and to cut ends of *Ruta graveolens* has been efficaciously and selectively converted into bergapten while no radioactivity was observed in the other isolated furocoumarins.

Linear furocoumarins, called also psoralens, are a well known group of substances wich show evident photosensitizing activity on several biological systems [1, 2]. The photosensitizing properties of psoralens are used for the photochemotherapy of some skin-diseases [3, 4]. Two natural furocoumarins are used in this therapy: 8-methoxypsoralen (**VIII**) (8-MOP, xanthotoxin) since many years and more recently 5-methoxypsoralen (bergapten, 5-MOP) (**VI**) [5].

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In these last ten years the biogenesis of linear furocoumarins has been widely investigated: in particular the general picture of the biogenetic pathway of these substances is reported in Fig. 1.

As reported in the scheme the biogenesis of O-alkylfurocoumarins can take place from psoralen (**IV**) through hydroxylation in 5-, in 8- or in both these positions and successive alkylation according to the classical pathway of Brown and Coworkers [6] and to our experimental findings [7, 8].

An alternative pathway, proposed by our group [9], that should be activated in plants and herbs where psoralen is accumulated to a valuable extent (*e. g.* in *Ruta graveolens* and in *Ficus carica*), suggests that hydroxylation occurs at the level of 4′,5′-dihydrofurocoumarinic precursor, namely marmesin (**III**), obtaining the hydroxymarmesins (**V**) or (**VII**), from which O-alkylderivatives can be formed.

This suggestion however has found experimental support only for 8-position and only in the case of *Ruta graveolens;* in particular we have observed that 8-methoxypsoralen is formed effectively from the product of hydroxylation of marmesin in 8-position (rutaretin) [9].

5-hydroxymarmesin was prepared for having further and possible definitive support of this second biogenetic pathway; in particular for having demonstration of the involvement of 5-position in the hydroxy-

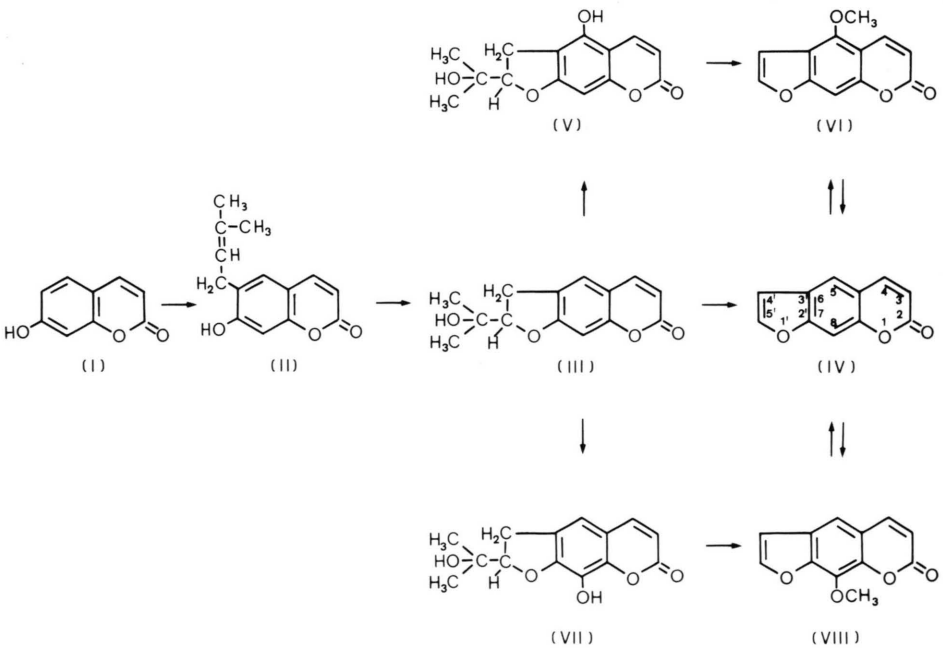

Fig. 1. General biogenetic pathway for linear furocoumarins.

Table I. Feeding experiments with labeled 5-hydroxymarmesin* on cut ends of *Ruta graveolens* and on leaves of *Ficus carica*.

Plant species	Recovered compounds					
	Psoralen		Bergapten		Xanthotoxin	
	specific activity**	dilution***	specific activity**	dilution***	specific activity**	dilution***
Ruta graveolens	0.006	118,000	3.04	215	0.004	168,000
Ficus carica	0.004	167,000	2.45	266	–	–

* Specific activity of administered 5-hydroxymarmesin was 6.53×10^7 DPM per μmol.
** Specific activity of recovered compounds is reported as DPM $\times 10^5$ per μmol.
*** Dilution is defined as the ratio between the specific activity of the compound administered and that of isolated furocoumarins.

lation processes of marmesin and moreover for testing this precursor in more than one plant or herb.

Chemical synthesis of 5-hydroxymarmesin (**V**) has been carried out in this Institute; this possible precursor of bergapten (**VI**) was adminstered to leaves of *Ficus carica* and to cut ends of *Ruta graveolens*. In the leaves of *Ficus carica* [10], in addition to psoralen (**IV**) bergapten (**VI**) is present too; in the cut ends of *Ruta graveolens* the main furocoumarins isolated are psoralen (**IV**), bergapten (**VI**) and xanthotoxin (**VIII**) [8].

The synthesis of 5-hydroxymarmesin (**V**) will be described in detail elsewhere; the compound has been labeled by the Radiochemical Centre (Amersham-England) by a catalytic exchange method (TR 8) and the purification has been carried out according to a procedure already described [11].

An aqueous solution of the labeled substance was administered to freshly cut leaves of *Ficus carica* and to cut ends of *Ruta graveolens* and the system was illuminated with a HWL Osram 500 W lamp. After the metabolism time (72 hours for fig-leaves and 96 hours for ruta), the fig-leaves and the cut ends of ruta were collected, dried and worked up to obtain the "coumarinic extracts" [11 – 13].

Psoralen (**IV**) and bergapten (**VI**) from *Ficus carica* and psoralen (**IV**), bergapten (**VI**) and xanthotoxin (**VIII**) from *Ruta graveolens* have been isolated and purified according to chromatographic procedure already described [14].

The isolated furocoumarins were eluted with ethanol and the solutions obtained utilized for spectrophotometrical measurements and for radiochemical determinations following a procedure described elsewhere [15].

The results obtained from feeding 5-hydroxymarmesin (**V**) to cut ends of *Ruta* (Table I) clearly show that this compound has been very effectively transformed into bergapten (**VI**), while isolated psoralen (**IV**) and xanthotoxin (**VIII**) did not show practically any incorporation of radioactivity. Analogous results have been obtained from fig-leaves; also in this case 5-hydroxymarmesin (**V**) has been converted only into bergapten (**VI**) while psoralen (**IV**) did not show incorporation of radioactivity.

These data demonstrate that 5-hydroxymarmesin (**V**) can be effectively and selectively converted into the corresponding O-alkylfurocoumarin (bergapten) (**VI**) both in *Ficus carica* and in *Ruta graveolens*, showing a behaviour strictly analogous to that previously observed with 8-hydroxymarmesin (rutaretin) (**VII**) [9].

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