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# BIOSYNTHESIS OF FURANOCOUMARINS IN PARSNIPS

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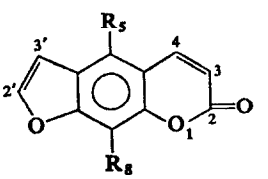
**Abstract**—Labelling experiments have shown that umbelliferone, and to a lesser degree *p*-coumaric acid, are precursors of bergapten, imperatorin, isopimpinellin and xanthotoxin in *Pastinaca sativa* L. C-2 and C-5 of mevalonate, which has been proposed as a precursor of two furan ring carbons and of dimethylallyl substituents, were both incorporated into furanocoumarins with low efficiency—poorer than that of acetate. Feeding experiments with [2-$^{14}$C]5-methoxy-7-hydroxycoumarin provided no evidence that it is a major intermediate in bergapten formation, supporting the theory that carbon side-chain attachment precedes further oxygenation of umbelliferone in furanocoumarin biosynthesis.

## INTRODUCTION

THE COMMON parsnip (*Pastinaca sativa* L.) contains a number of linear furanocoumarins, located primarily in the flowers and seeds of the plant.[1,2] By means of reciprocal grafting, Beyrich[3] established that furanocoumarins in this species are formed in the fruits, and found no evidence for their subsequent translocation. Beyrich's experiments on the time course of their biosynthesis[2] revealed a sharp rise in the content of bergapten, imperatorin, isopimpinellin, and xanthotoxin (I) during the flowering period and early stages of fruit formation. Little appears to be known about the reaction sequences of furanocoumarin formation in this species. However, information has been reported on the nature of the process in other umbellifers[4-8] which shows that umbelliferone and, for the linear isomers, marmesin are intermediates, and suggests that mevalonate may be the source of carbons 2′ and 3′.




STRUCTURE I.

Bergapten: $R_5 = OCH_3$, $R_8 = H$.
Imperatorin: $R_5 = H$, $R_8 = OCH_2—CH=C(CH_3)_2$.
Isopimpinellin: $R_5 = R_8 = OCH_3$.
Xanthotoxin: $R_5 = H$, $R_8 = OCH_3$.

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[2] T. BEYRICH, *Pharmazie* **6**, 365 (1966).
[3] T. BEYRICH, *Planta Med.* **15**, 306 (1967).
[4] H. G. FLOSS and U. MOTHES, *Phytochem.* **5**, 161 (1966).
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[6] W. STECK, S. A. BROWN and M. EL-DAKHAKHNY, *Tetrahedron Letters* 4805 (1969).
[7] S. A. BROWN, M. EL-DAKHAKHNY and W. STECK, *Can. J. Biochem.* (in press).
[8] W. STECK and S. A. BROWN, *Can. J. Biochem.* (in press).

## RESULTS AND DISCUSSION

In the experiments described here, the utilization of several possible precursors of furanocoumarins has been investigated in parsnips during the period of rapid synthesis. The four principal furanocoumarins were recovered and analysed for $^{14}C$, and the results are collected in Table 1. The data for imperatorin are incomplete, owing to failure to obtain satisfactorily pure samples in several cases. (Imperatorin could not be purified by GLC because of decomposition on the column.)

The most efficient precursor of these four coumarins, taken as a whole, is clearly [2-$^{14}C$]umbelliferone. This observation is consistent with those already made in the umbellifers *Pimpinella magna*,[4] *Heracleum lanatum*,[7,8] and *Angelica archangelica*,[8] and in *Ruta graveolens*,[7] and is in accord with the theory that all furanocoumarins originate from this intermediate. [2-$^{14}C$]*p*-Coumaric acid was also moderately well utilized in the synthesis of all four coumarins, as would have been anticipated from its known function as a precursor of 7-oxygenated benzpyrones in other species.[9]

[2-$^{14}C$]5-Methoxy-7-hydroxycoumarin was administered to test the theory of Floss and Mothes[4] that further oxygenation of the aromatic ring of umbelliferone precedes attachment of the side-chain involved in the elaboration of the furan ring. More recent evidence has favoured the reverse order of substitution.[5,7] The results from the present experiments are somewhat puzzling. In the one case where a satisfactory imperatorin sample was recovered, it had the highest specific activity of any of the coumarins, in fact higher than the imperatorin from the umbelliferone feeding. Bergapten, which might have been expected to accumulate $^{14}C$ in these feedings, was only moderately labelled, much less than after the umbelliferone feeding. Any explanation centering around demethoxylation of the 5-methoxy-7-hydroxycoumarin to umbelliferone encounters the objection that neither xanthotoxin nor isopimpinellin was appreciably labelled. The only conclusion possible at present seems to be that there is no evidence for any major pathway to bergapten involving 5-methoxy-7-hydroxycoumarin.

The remaining feedings were intended to elucidate further the role of mevalonate in furanocoumarin biosynthesis. Existing evidence has come from two laboratories. Caporale, Breccio and Rodighiero[10] compared [2-$^{14}C$]mevalonic acid with several other compounds, including [2-$^{3}H$]acetate, as precursors of bergapten and psoralen in *Ficus carica*, with a 72-hr metabolic period. Mevalonate was utilized for furanocoumarin biosynthesis more than thirty times as well as acetate, judged both by dilution values and per cent incorporation. The dilution was, nevertheless, over 1000 for mevalonate, and it is difficult to reconcile the incorporation of C-2 of mevalonate into the furan ring with known pathways of this compound's metabolism. In a later study, Floss and Mothes[4] recovered five labelled furanocoumarins after administration of [4-$^{14}C$]mevalonic acid (as the dibenzylethylenediamine salt) to *Pimpinella magna* for 14 days, and concluded after degradation of pimpinellin that the label was concentrated in C-2′ of the furan ring, as would have been predicted. Carboxyl-labelled cinnamic acid was fed for comparison, and its incorporation was of the same order as that of mevalonate, but in all cases high dilutions of about 6000–90,000 were observed.

The data of Table 1 include the results of administrations of [2-$^{14}C$]- and [5-$^{14}C$]mevalonate and of [1-$^{14}C$]- and [2-$^{14}C$]acetate, in addition to [2-$^{14}C$]umbelliferone. The conclusions

[9] S. A. BROWN, in *Biosynthesis of Aromatic Compounds* (edited by G. BILLEK), p. 15, Pergamon Press, Oxford (1966).

[10] G. CAPORALE, A. BRECCIO and G. RODIGHIERO, *Prepn. Bio-Med. Appl. Labeled Mol., Proc. Symp.*, p. 103, Venice (1964).

TABLE 1. CONVERSION OF LABELLED COMPOUNDS TO FURANOCOUMARINS IN *Pastinaca sativa*

| Compound fed | | | | Compounds isolated* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Imperatorin | | Bergapten | | Xanthotoxin | | Isopimpinellin | |
| Name | Spec. act. μc/mmole | Total, μmoles | Dose, μmole/g fr. wt. | Spec. act. | Dilution | Spec. act. | Dilution | Spec. act. | Dilution | Spec. act. | Dilution |
| [2-$^{14}$C]Umbelliferone | 99·5 | 200 | 1·2 | 1·96 | 51 | 6·73 | 15 | 7·12 | 14 | 1·97 | 51 |
| [2-$^{14}$C]*p*-Coumaric acid | 80·9 | 178 | 0·90 | 0·24 | 286 | .. | .. | 0·33 | 208 | 0·13 | 529 |
| [2-$^{14}$C]*p*-Coumaric acid | 99·9 | 200 | 0·93 | — | — | 0·30 | 265 | 2·01 | 40 | 0·79 | 101 |
| [2-$^{14}$C]5-Methoxy-7-hydroxycoumarin | 100 | 200 | 0·88 | 1·78 | 42 | 0·66 | 113 | 0·005 | 15,000 | 0·015 | 5,000 |
| [2-$^{14}$C]5-Methoxy-7-hydroxycoumarin | 100 | 200 | 1·1 | — | — | 0·37 | 215 | 0·007 | 11,000 | 0·027 | 2,900 |
| [1-$^{14}$C]Sodium acetate | 97 | 200 | 0·95 | 0·05 | 1,500 | 0·15 | 520 | 0·035 | 2,200 | 0·05 | 1,500 |
| [1-$^{14}$C]Sodium acetate | 97 | 200 | 0·83 | 0·09 | 800 | 0·02 | 3,500 | 0·02 | 3,500 | 0·05 | 1,500 |
| [2-$^{14}$C]Sodium acetate | 86 | 200 | 0·83 | — | — | 0·025 | 2,400 | 0·03 | 2,000 | 0·025 | 2,500 |
| [2-$^{14}$C]Mevalonic acid lactone | 103 | 196 | 0·79 | 0·045 | 1,500 | 0·18 | 390 | 0·005 | 15,000 | 0·025 | 3,000 |
| [2-$^{14}$C]Mevalonic acid lactone | 103 | 196 | 1·0 | 0·07 | 1,500 | 0·10 | 900 | 0·004 | 25,000 | 0·015 | 6,000 |
| [5-$^{14}$C]Mevalonic acid lactone | 88 | 196 | 0·77 | — | — | 0·006 | 10,000 | 0·009 | 6,500 | 0·08 | 800 |
| [5-$^{14}$C]Mevalonic acid lactone | 88 | 196 | 0·67 | 0·035 | 1,500 | 0·025 | 2,000 | 0·009 | 6,000 | 0·02 | 2,500 |

* Spec. act., μc/m mole. Dilutions are corrected for differences in plant size by multiplying calculated dilution by a factor = wt. of plant fed umbelliferone/wt. of plant.

.. Sample lost.

emerging from this set of data are (1) in comparison to umbelliferone, both mevalonate and acetate are very poorly utilized in furanocoumarin biosynthesis, (2) in general, mevalonate appears to be more poorly utilized than acetate, and (3) there is no appreciable difference in the incorporation of C-2 and C-5 of mevalonate. It was not feasible to degrade the recovered coumarins owing to the low total activity recovered, but in the context of the low utilizations of mevalonate and acetate the value of such degradations would be questionable in any event. The greater number of metabolic pathways open to mevalonate compared to umbelliferone, and possibilities of compartmentalization, make the former's higher dilution values no surprise. But the differences of two or more orders of magnitude observed here, coupled with the poorer utilization of mevalonate (especially the [5-$^{14}$C]-labelled species) relative to acetate, raise significant doubts about the role of mevalonate in furanocoumarin formation, at least in parsnips. It should also be noted that the specific activities of imperatorin, a dimethylallyl ether, were of the same order as those of coumarins lacking the dimethylallyl residue. This is in agreement with the finding of Kunesch and Polonsky[11] on the origin of this group in calophyllolide, a coumarin of *Calophyllum inophyllum*. Another instance of poor incorporation of mevalonate relative to acetate was reported by Hamada and Chubachi[12] from their studies on the origin of the dihydrofuran ring of rotenone. In spite of a specific incorporation ratio of only 0·007% ([2-$^{14}$C]acetate gave 0·064) these workers concluded from the results of degradation that C-7′ and C-8′ of rotenone are derived from C-2 of mevalonic lactone.

While the work of Floss and Mothes[4] leaves little doubt that mevalonate is convertible to furanocoumarins, consideration of other possible origins for the 3-methylbutanoid moiety of marmesin is indicated in the light of the repeated failures to achieve anything but low incorporations of [5-$^{14}$C] mevalonate relative to acetate and, especially, to [2-$^{14}$C]mevalonate. Experiments aimed at further elucidation of this question are continuing.

## EXPERIMENTAL

### *Cultivation and Feeding of Plants*

Parsnips were started from seed outdoors in the spring and moved to a greenhouse in the early autumn. Flowering began in April of the following year and continued until June. Labelled compounds were administered to various plants over about a 1-month period. The first dose was given when fruits of the first umbel to flower were *ca.* 5 mm long, and fruit formation was proceeding on at least one other umbel, with still other umbels in various stages of flowering. The labelled compound was dissolved in 3 ml of water, with the aid of an equivalent of $NaHCO_3$ for *p*-coumaric acid or of NaOH for the phenolic coumarins. Two small holes were pierced in the upper end of an internode on the main flower stalk, and a quantity of the solution was injected from a syringe through one of them into the hollow interior. The solution was divided among three internodes—the highest ones with enough volume to accommodate it. After 2 days a flush of 3 ml of water was similarly injected. A second dose and flush was given 7 days after the first. The plant was harvested 30 days after the first injection.

### *Isolation and Analysis of Coumarins*

Since preliminary investigation revealed no significant radioactivity in the roots, only the aerial organs were extracted. These varied in fr. wt. from 170 to 291 g, with an average of 222 g. The procedure for isolation and resolution of the coumarin fraction has been described elsewhere.[7] After preliminary purification on formamide-impregnated Silica Gel H (Merck), final separation was accomplished by GLC,[13] except in the case of imperatorin, which was simply sublimed *in vacuo* at 110°. Identity of the bands from these treatments was confirmed with the aid of u.v. spectra, which also served for quantitative analysis. Radioactivity was measured in Bray's solution with a Nuclear-Chicago Corp. model 6822 scintillation spectrometer.

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[13] W. STECK and B. K. BAILEY, *Can. J. Chem.* **47**, 3577 (1969).

*Labelled Compounds*

Labelled acetate and mevalonate were obtained from commercial sources. Preparations of labelled *p*-coumaric acid,[14] umbelliferone[15] and 5-methoxy-7-hydroxycoumarin[7] have been reported elsewhere.

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