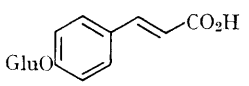
"Anomalous" Results in Biosynthetic Studies with Multiply-labelled Precursors

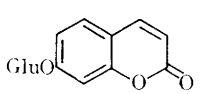
By D. J. AUSTIN* and M. B. MEYERS†

(Department of Chemistry, University of Glasgow, Glasgow, W.2)

"MULTIPLE-LABEL" biosynthetic studies usually involve the administration to biological systems of precursor molecules labelled at various sites with the same or different isotopic species in known proportion. An evaluation of the corresponding isotopic proportion in the reaction product can indicate whether the precursor has been incorporated into the product without separation of the labelled parts.



(I)



(II)

In extension of our previous results,[1] this report describes feedings of *trans-p-β*-D-glucosyloxycinnamic acid (I) (2-[^{14}C]- and U-[^{14}C]-glucose-labelled) to *Hydrangea macrophylla* Ser. followed by measurement of the chain and glucose radioactivity present after one, three, and five days in the skimmin (II) formed by this plant.

TABLE 1

*Percentage incorporations into skimmin**

	1 Day	3 Days	5 Days
2-[^{14}C]inc.	3·05	4·12	4·28
U-[^{14}C]glucose inc...	0·20	2·51	3·94
Relative inc. glu/chain	6·7%	61·0%	92·2%

* Dose = 12 μmoles to each plant, 2-[^{14}C]-activity = $1{\cdot}07 \times 10^6$ c.p.m./mmole, U-[^{14}C]glucose activity = $1{\cdot}04 \times 10^6$ c.p.m./mmole.

The results presented in Table 1 show that, as previously established, the chain label of the fed glucoside is consistently very well incorporated into skimmin. However, after one day the utilisation of the glucose label is only some 7% relative to that of the chain, demonstrating that a

* Present address: Department of Chemistry, University of California, Los Angeles, 24.
† Present address: Department of Chemistry, College of Technology, Belfast, 1.

hydrolysis of the glycosyl linkage has taken place at some stage. This apparently unambiguous finding, which is consistent *either* with an oxidation of the aglycone (perhaps in "activated" form) *or* with a delayed hydrolysis, is not reinforced by the three- and five-day results. On the contrary, the latter accords with an incorporation of the glucoside *without* hydrolysis.

These data, although self-contradictory at first sight, can be rationalised in terms of fragmentation of the doubly-labelled precursor with differential uptake of the products into skimmin.

As much as 14% of the *trans-p-*β-D-glucosyloxycinnamic acid fed to hydrangea is hydrolysed after one day[2] and, at this dose level, free *trans-p*-coumaric acid is rapidly transformed to skimmin.[1] It is suggested that in the present experiments the [^{14}C]glucose liberated by hydrolysis has not been significantly incorporated until the bulk of the endogenous *unlabelled* pool of glucosylation reagent (presumably UDP-glucose[3]) has interacted with a 2-[^{14}C]labelled precursor of skimmin. This concept is supported by the experimental fact (Table 2) that the relative amounts of glucose radioactivity found in the skimmin formed during the successive metabolic periods rise from a level 0·07 to one almost 9 times that of the corresponding chain activity.

TABLE 2

Successive increments in percentage incorporation

	1st Day	2nd—3rd Days	4th—5th Days
2-[^{14}C]-inc.	3·05	1·07	0·16
U-[^{14}C]glucose inc. . .	0·20	2·31	1·43
Ratio glu/chain . .	0·07	2·16	8·93

Alternative interpretations are that the [^{14}C]-glucose has been incorporated by reaction with preformed umbelliferone or skimmin (by exchange with the unlabelled glycosyl group).

The available evidence is insufficient to permit a precise definition of the pathway. In any event, after a lag period of one day, the liberated [^{14}C]glucose has been much more efficiently incorporated into skimmin than the 2-[^{14}C]-*p*-coumaric acid. After five days, this has fortuitously yielded the gross effect of an *en bloc* utilisation of the glucoside.

This interpretation of the five-day result is directly contrary to the tacit assumption of multiple-label experimentation—that an observed retention of the initial isotopic proportion constitutes sufficient proof of the incorporation of the fed precursor *as such*. This assumption is justified only when at least one of the endogenous metabolic pools of the precursor breakdown products is large enough to cause a significant dilution of activity in the event of recombination (*cf.* the present one-day result). If this is not the case, or if the rates of uptake of the breakdown products are sufficiently different during the metabolic period, then the observed overall dilutions of the products may be similar. Recombination at some stage will therefore give rise to a misleading result. We feel that the "anomalous" results presented here constitute a clear-cut example of the operation of such a differential recombination process.

This problem in interpretation has been observed in a particularly favourable case but analogous situations with other labile precursors (*e.g.*, *O*- or *N*-methylated) may well occur. Suitable controls, such as time-course or *in vitro* enzymic studies, could prove valuable in deciding the true operating pathways.

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[1] D. J. Austin and M. B. Meyers, *Phytochemistry*, 1965, **4**, 245, 255.
[2] D. J. Austin and M. B. Meyers, unpublished results.
[3] J. B. Pridham, *Phytochemistry*, 1964, **3**, 493; J. B. Pridham, *Ann. Rev. Plant Physiol.*, 1965, **16**, 13.