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ORIGIN OF THE METHYLENEDIOXY CARBON IN PHLEBIARUBRONE: FORMATE AND METHIONINE AS PRECURSORS

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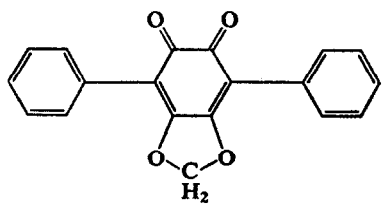
Abstract—The relative efficiency of methionine and formate as precursors of the methylenedioxy carbon of phlebiarubrone has been investigated using methionine-Me-^{14}C and formate-^{13}C. When the substrates are added separately, incorporation is similar for both. When methionine and formate are added together, the incorporation of formate is markedly reduced.

PHLEBIARUBRONE (I), an orthoquinone produced by the basidiomycete *Phlebia strigosozonata*, has a terphenyl moiety and a methylenedioxy group.[1] Incorporation of ^{13}C-labelled phenylalanine into the terphenyl ring system was reported recently.[2] Determination by mass spectroscopy of the relative proportions of singly and double labelled I made it possible to calculate the amount of endogenous phenylalanine available for the biosynthesis of I. We now report the use of simultaneous addition of different substrates, one labelled with ^{13}C, the other with ^{14}C, to determine their relative efficiency as precursors for the methylenedioxy group of I



(I)

Labelled formate (^{13}C or ^{14}C) was incorporated efficiently (Table 1), and exclusively in the methylene dioxy ring (Table 2). As much as 30% of the carbon of this group was derived from the added formate, depending on the amounts added. Results with methionine (^{14}C) were similar (Tables 1 and 2). When formate and methionine were added together, the incorporation of methionine was not significantly affected, but that of formate was drastically reduced to a degree dependent on the proportions of the two precursors added (Table 3).

[1] T. C. MCMORRIS and M. ANCHEL, *Tetrahedron Letters* 335 (1963).
[2] A. K. BOSE, K. S. KHANCHANDANI, P. T. FUNKE and M. ANCHEL, *Chem. Commun.* 1347 (1969).

TABLE 1. EFFICIENCY OF INCORPORATION* INTO PHLEBIARUBRONE, OF LABELLED FORMATE OR METHIONINE ADDED SEPARATELY TO CULTURES OF *Phlebia strigosozonata*

Experiment	Precursor	mM/flask	Specific activity (mc/mM or atom % excess)		Efficiency of incorporation* (%)
			Precursor	Phlebiarubrone	
1	Formate-^{14}C	0·15	0·034	0·006	18
	Formate-^{13}C		55	11	20
2	Formate-^{14}C	0·15	0·034	0·006	18
	Formate-^{13}C		55	11	20
3	Formate-^{14}C	0·15	0·034	0·0053	16
	Formate-^{13}C		55	11	20
4	Formate-^{14}C	0·30	0·017	0·005	30
	Formate-^{13}C		27·5	8	29
5	Methionine-Me-^{14}C	0·017	0·099	0·0047	4·6
6	Methionine-Me-^{14}C	0·033	0·05	0·0028	5·7
7	Methionine-Me-^{14}C	0·17	0·0099	0·0018	18

* Efficiency of incorporation $= \frac{\text{mc/mM of product}}{\text{mc/mM of precursor}} \times 100.$

TABLE 2. DISTRIBUTION OF LABEL FROM FORMATE OR METHIONINE IN PHLEBIARUBRONE

Precursor	Phlebiarubrone leucoacetate (mc/mM)	Polyporic acid leucoacetate (mc/mM)	Formaldehyde dimedon derivative (mc/mM)
Formate-^{14}C	15·8	0·045	18·5
Methionine-Me-^{14}C	2·8	0·007	3.0

Examination of Table 1 reveals that formate or methionine alone, when added at similar levels, show a similar efficiency of incorporation (cf. Experiments 1–3, 7). As expected, this increases with the amount of precursor added (cf. Experiments 5 and 7, and Experiments 1 and 4). For the amounts of precursor used in these experiments, the curve of efficiency against mM of precursor added is almost a straight line, suggesting that the availability of the C-1 donor is still limiting.

When formate and methionine were added to the same flask, and the proportion of formate to methionine varied from 5-fold to 135-fold (Experiments 10–13) the efficiency of incorporation *per mole* of methionine, was about four times that of formate (last column, Table 3). However, when the two substrates were added in equimolar amounts (Experiments 8 and 9), the incorporation of formate was below the amount measurable by the mass spectrometer (about 1% enrichment). Had the same relationship as in Experiments 10–13 held in Experiments 8 and 9, the formate should have been incorporated with an efficiency of about 5%.

The results are compatible with a sequence in which the labelled carbon of formate enters phlebiarubrone by way of methionine; but when a greater amount of methionine is present, as in Experiments 8 and 9, the transfer of the carbon to homocysteine to form methionine is repressed. Under these conditions the label from formate would not appear in phlebiarubrone. This would account for the consistently better incorporation of methionine, and also the failure of formate to incorporate in Experiments 8 and 9, in which the level of methionine

TABLE 3. EFFICIENCY OF INCORPORATION* INTO PHLEBIARUBRONE, OF LABELLED FORMATE AND METHIONINE, ADDED TOGETHER TO CULTURES OF *Phlebia strigosozonata*

Experiment	Precursor	mM/flask	Ratio M/F	Specific activity (mc/mM or atom % excess)		Efficiency of incorporation*	Ratio: Efficiency/mole M / Efficiency/mole F
				Precursor	Phlebiarubrone		
8	Methionine-Me-^{14}C	0·13	1	0·006	0·0016	26	>13
	Formate-^{13}C	0·13	1	58	<1	<2	
9	Methionine-Me-^{14}C	0·15	1	0·045	0·0093	21	>10
	Formate-^{13}C	0·15	1	58	<1	<2	
10	Methionine-Me-^{14}C	0·03	1	0·0266	0·0032	12	3·7
	Formate-^{13}C	0·15	5	58	9	16	
11	Methionine-Me-^{14}C	0·015	1	0·057	0·0026	4·5	4·1
	Formate-^{13}C	0·12	8	58	5	9	
12	Methionine-Me-^{14}C	0·015	1	0·05	0·0027	5·1	3·4
	Formate-^{13}C	0·15	10	58	10	18	
13	Methionine-Me-^{14}C	0·0011	1	11·52	0·04	0·3	4·5
	Formate-^{13}C	0·15	135	58	5	9	

* Efficiency of incorporation $= \frac{\text{mc/mM of product}}{\text{mc/mM of precursor}} \times 100.$

was 5–100-fold that in Experiments 10–13. To test the validity of this explanation, it would be necessary to add the substrates in equal amounts but at a lower level. Unfortunately, at the low level required, the expected incorporation of formate-^{13}C would be below that determinable by mass spectroscopy. It should be possible to carry out this experiment by reversing the isotopic labelling, i.e. by use of formate-^{14}C and methionine-Me-^{13}C. The latter is not, at present, commercially available.

EXPERIMENTAL

The labelled substrates used were sodium formate-^{14}C (spec. act. 42·8 mc/mM, Nuclear Chicago), sodium formate-^{13}C (55% enrichment), and methionine (methyl-^{14}C, 11·52 mc/mM, Calbiochem., or 56·8 mc/mM, Amersham). These were diluted with appropriate amounts of the corresponding "cold substrate" to the desired number of moles. The isotopic precursor was added to 3-week-old shake cultures in which pigment formation had begun. Each 125 ml flask contained 40 ml of 2% malt extract, inoculated with *Phlebia strigosozonata*. Two weeks later the phlebiarubrone was harvested and converted to the acetate.[1] Incorporation of ^{13}C was determined by mass spectrometry and location of the isotope in the molecule was determined by NMR as previously described.[2] Incorporation of ^{14}C was determined in a liquid scintillation counter (Nuclear Chicago, 6810 Liquid scintillation system). The position of the radioactive label was determined by cleavage of the methylene dioxy ring, and conversion of the formaldehyde so obtained, to the dimedon derivative,[1] the form in which it was counted.

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