Stages in the Biosynthesis of [6]-Gingerol in *Zingiber officinale*

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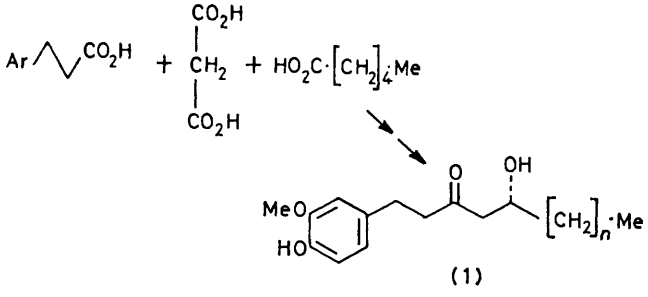
Summary [2-^{14}C,^{3}H,3-^{3}H]Dihydroferulic acid and [1-^{14}C, 6-^{3}H]hexanoic acid are shown to be incorporated into (*S*)-[6]-gingerol in *Zingiber officinale*, the ^{3}H/^{14}C ratio falling by $\frac{1}{2}$ in the first case but remaining constant in the second; a biogenetic scheme is proposed to accommodate these results, and includes discussion of the late stages of biosynthesis in the light of satisfactory incorporation of [10-^{3}H]-[6]-dehydrogingerol (**5**).

THE pungent rhizome of *Zingiber officinale* Roscoe (ginger) contains the phenols (**1**; n = 1, 2, 3, 4, 6, 8, and 10) of which [6]-gingerol[1] (**1**; n = 4) is the major component. We have described previously[2] experiments on the biosynthesis of [6]-gingerol, showing that phenylalanine, *p*-coumaric, ferulic, and dihydroferulic acids are incorporated into Ar–C(1)–C(3), that [2-^{14}C]acetate supplies C(4) efficiently, and that [1-^{14}C]hexanoate provides C(5)–C(10) with the label intact at C(5). These results favoured the basic assembly pattern of Scheme 1. We now report further definition and modification of this pathway.



SCHEME 1.

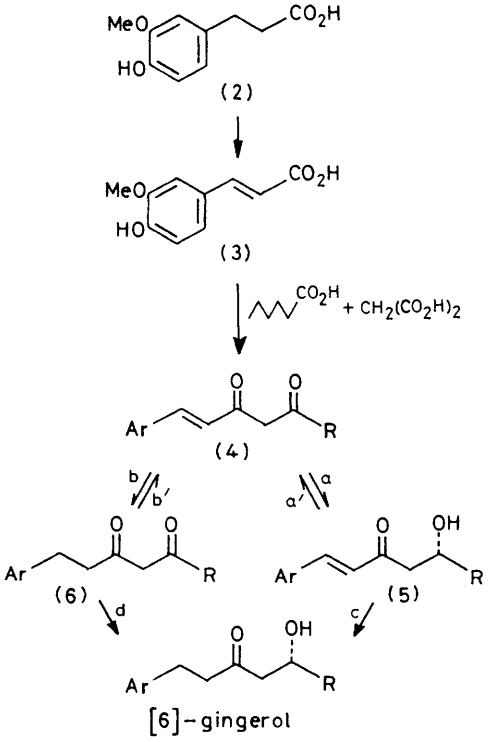
The role of dihydroferulic acid (**2**) was examined first, using dual-labelling. Sodium [2-^{14}C,^{3}H,3-^{3}H]dihydroferulate {prepared from vanillin and [2-^{14}C]malonic acid, followed by reduction of the [2-^{14}C]ferulate in a modified Brown apparatus with tritium-enriched deuterium, to minimise unfavourable kinetic isotope effects} was wick-fed

to shoots of whole ginger plants,† during the period of rhizome growth. [6]-Gingerol was isolated and its methyl ether recrystallised to constant activity. The $^3H/^{14}C$ ratio was measured in both precursor and metabolite, and the results (Table 1) show that 47% of the tritium was lost during incorporation of the dihydroferulate. It is thus implied that dihydroferulate is dehydrogenated *in vivo* to ferulate before elaboration to [6]-gingerol: if as expected the enzymatic dehydrogenation is stereospecific, 50% loss of tritium is predicted, whether *cis* or *trans* vicinal hydrogens are removed, and regardless of the stereochemical distribution of the 2,3-tritiums in the racemic precursor.

TABLE 1. Isotope ratios for administration of [^{14}C,^3H]precursors to [6]-gingerol in *Z. officinale*.

Precursor	(A) $^3H/^{14}C$ (Precursor)	(B) $^3H/^{14}C$ (6-Gingerol)	B/A
[2-^{14}C,^3H,3-^3H]Dihydroferulate	18·8	9·9	0·53
[1-^{14}C,6-^3H]Hexanoate	6·6	6·8	1·03

A similar experiment was carried out with sodium [1-^{14}C,6-^3H]hexanoate. The latter was prepared from 6-iodohexanol *via* decomposition with tritiated water of the Grignard reagent from its trimethylsilyl ether, and oxidation of the resulting [6-^3H]hexanol to the acid. The results of an incorporation experiment in ginger plants are shown in Table 1 and clearly demonstrate the utilisation of intact hexanoate in the biosynthesis.



SCHEME 2.

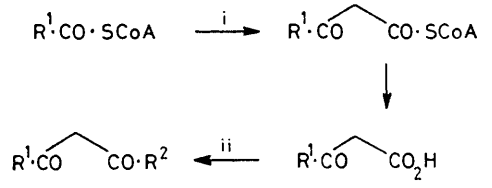
Thus the assembly of the carbon skeleton of [6]-gingerol involves hexanoate, malonate, and ferulate rather than dihydroferulate (Scheme 2). Such a condensation requires [6]-dehydrogingerdione (**4**) as initial product; two reduction steps are then necessary to transform (**4**) to [6]-gingerol, and either [6]-dehydrogingerol (**5**) or [6]-gingerdione (**6**) must be an intermediate. [10-^3H]Samples of (**4**), (**5**), and (**6**) were synthesised[3] from [6-^3H]hexanoic acid, and applied to whole ginger plants: radiochemical results are given in Table 2. Examination of Scheme 2 suggests close

TABLE 2. Administration of [^3H]precursors to *Z. officinale*

Precursor	Specific incorporation /%	Dilution
[10-^3H]Dehydrogingerdione (**4**)	0·009	$1·1 \times 10^4$
[10-^3H]Dehydrogingerol (**5**)[a]	0·12	$8·5 \times 10^2$
[10-^3H]Gingerdione (**6**)	0·004	$2·6 \times 10^4$

[a] Corrected for incorporation of a single enantiomer.

similarities between the C=O reduction steps (a) and (d), which could even be mediated by the same, not completely substrate-specific, enzyme system; the same point could be made about the two C=C reductions (b) and (c), and again about the dehydrogenations (**2**) → (**3**) and (b′). Not unexpectedly, then, all three compounds (**4**)—(**6**) are fairly well incorporated; compared to the first-formed diketone (**4**), dehydrogingerol shows a higher incorporation (lower dilution) while the reverse is true for gingerdione (**6**). This evidence suggests, *supra facie*, that, in whole plants, (**5**) is the preferred intermediate, with (a)—(c) the major path, while (**6**) is involved either in a slower reduction (d), or in the (b′)—(a)—(c) sequence. However this conclusion must be treated with caution in view of the differences between individual plants and uncertainties over pool sizes, and further experiments are required to elucidate the workings of this grid.



SCHEME 3. Reagents i $HO_2C·CH_2·CO·SCoA$, ii $R^2CO·SCoA$.

The condensation of Scheme 2 must follow the general course of Scheme 3, where $R^1,R^2 = ArCH_2CH_2$ and $Me-[CH_2]_4$ in an undetermined order. This type of 'biological Claisen' reaction, in which the malonate component loses both carboxy-groups, is novel, and although postulated some time ago[4] has been shown experimentally to operate only in the present case and that of 9-phenylphenalenones, *e.g.* in *Lachnanthes tinctoria*.[5]

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[1] D. W. Connell and M. D. Sutherland, *Austral. J. Chem.*, 1969, **22**, 1033.
[2] P. Denniff and D. A. Whiting, *J.C.S. Chem. Comm.*, 1976, 711.
[3] P. Denniff and D. A. Whiting, *J.C.S. Chem. Comm.*, 1976, 712.
[4] R. Thomas, *Biochem. J.*, 1961, **78**, 807. See also R. Thomas, *Chem. Comm.*, 1971, 739 and P. J. Roughley and D. A. Whiting, *J. Chem. Soc.*, 1973, 2379 for further discussion.
[5] J. M. Edwards and R. J. Highet, *Tetrahedron Letters*, 1977, 4471.