Biosynthesis of [6]-Gingerol, Pungent Principle of *Zingiber Officinale*

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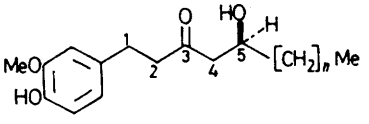
Summary Evidence is adduced for a scheme of biosynthesis for (*S*)-(+)-[6]-gingerol in *Zingiber officinale* Roscoe, in which dihydroferulate is built up from phenylalanine and participates in a new example of a 'biological Claisen reaction' with malonate and hexanoate; the β-diketone product is then reduced to [6]-gingerol.

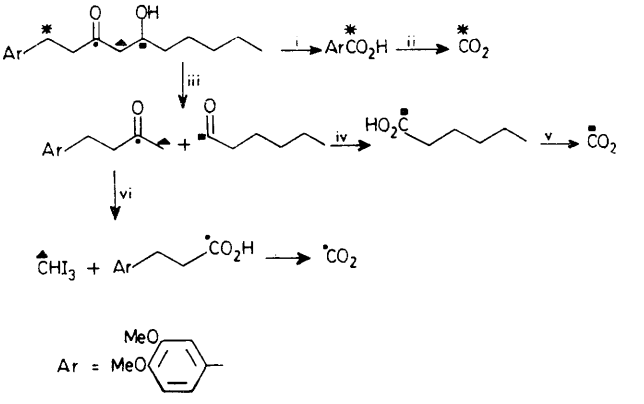
GINGER, the rhizome of *Zingiber officinale* Roscoe (Zingiberaceae), has been valued since antiquity for its flavour and pungent qualities. Although the oleoresin containing the pungent principles has been subject to many chemical investigations over more than a century, the sensitive



(1) *n* = 4, (*S*)-(+)-[6]-Gingerol
(2) *n* = 6, [8]-Gingerol
(3) *n* = 8, [10]-Gingerol

organoleptic compounds have only recently been identified. The major constituent is (*S*)-(+)-[6]-gingerol (**1**),[1] accompanied in the plant by a series of homologues[1,2] *e.g.* [8]- and [10]-gingerols (**2**) and (**3**), and relatives with simple functional variances.

The biosynthesis of [6]-gingerol appears on general inspection to involve cinnamate–acetate condensations; various assembly routes are possible. The elucidation of the pathway is not only of interest *per se* but in connection with the many natural phenolics with related biogenesis (*e.g.* stilbenes, diarylheptanoids,[3] benzophenones, *etc.*), and here we report our results on this problem.



SCHEME 1. Degradation of [6]-gingerol. *Reagents:* i, $KMnO_4$; ii, heat, Cu, quinoline; iii, OH^-; iv, Ag_2O; v, $Pb(OAc)_4$, LiCl; vi, KI, I_2, OH^-.

Cultivated ginger plants† were employed in standard experiments with labelled precursors; the latter (Tables 1 and 2) were wick-fed to shoots during the period of rhizome extension. (*S*)-(+)-[6]-Gingerol was isolated and purified by crystallisation of its *O*-methyl ether, which, when

TABLE 1

Administration of labelled precursors to *Z. officinale*.

Precursor	Incorporation		Fractional distribution of label			
			1 2 3	4	5	6—10
			$ArCH_2{\cdot}CH_2{\cdot}CO$	CH_3	$CH(OH)$	C_5H_{11}
(±)-[1-^{14}C]-Phe	0·009	$1{\cdot}16\times10^7$	0·89	0	0	0
L-[*U*-^{14}C]-Phe	0·006	$1{\cdot}42\times10^8$	0·99	0	0	0
[2-^{14}C]-$MeCO_2Na$	0·011	$1{\cdot}84\times10^6$	0·05	0·49	0·03	0·43
[1-^{14}C]-$MeCO_2Na$	0·002	$9{\cdot}94\times10^6$	0·29		0·46	0·26
[1-^{14}C]-n-$C_5H_{11}{\cdot}CO_2Na$	0·017	$2{\cdot}22\times10^6$	0·30		0·70	0
[1-^{14}C]-n-$C_5H_{11}{\cdot}CHO$	$<10^{-7}$					

† We thank the Tropical Products Institute for viable Carribean ginger roots.

appropriate, was degraded using the reactions of Scheme 1 to establish labelled atom distribution. The results are listed in the Tables.

(L)-[*U*-^{14}C]- and (±)-[1-^{14}C]-Phenylalanine are both incorporated into [6]-gingerol at levels considered significant in experiments with whole plants; degradations show the label(s) to be contained within the Ar–C_3 section of the molecule. [^{3}H]-*p*-Coumaric acid (**4**) and [^{3}H]ferulic acid[3] (**5**) were both better precursors than Phe. The (relatively) high incorporation of ferulic acid may reflect an experimental difference; only actively developing rhizomes were fed and harvested in this case. [^{3}H]Dihydro-*p*-coumaric acid was a poor precursor, but [^{3}H]dihydroferulic acid (**6**) was utilised at an acceptable level. With variable product yields, dilution is a more reliable guide to precursor involvement than incorporation; thus dihydroferulic acid is diluted much less than *p*-coumaric acid although the incorporations do not differ greatly. It therefore appears that the reduction of the double bond in ferulic acid is a relatively early stage (preceding further condensations) in gingerol biosynthesis; the stereochemistry of this reaction is under examination.

TABLE 2

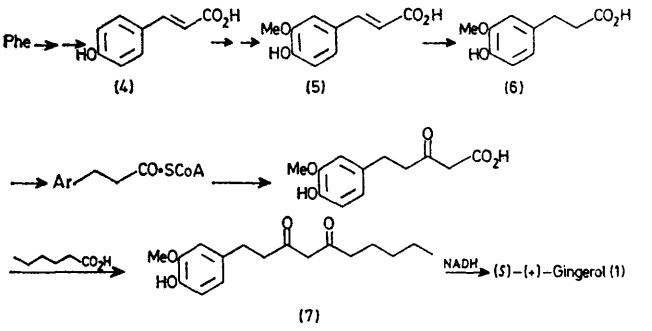
Administration of labelled precursors to *Z. officinale*

Precursor	Incorporation	Dilution
[^{3}H]Dihydro-*p*-coumaric acid	0·004	$7{\cdot}08 \times 10^4$
[^{3}H]-*p*-Coumaric acid (**4**)	0·062	$2{\cdot}44 \times 10^4$
[^{3}H]Ferulic acid[a] (**5**)	0·249	$5{\cdot}94 \times 10$
[^{3}H]Dihydroferulic acid (**6**)	0·033	$6{\cdot}88 \times 10^2$

[a] In this experiment only developing, not total, rhizomes were used.

Both sodium [1-^{14}C]- and [2-^{14}C]-acetate were incorporated into [6]-gingerol. With methyl labelled acetate very little radioactivity was found in the Ar–C_3 unit or at C-5, but the majority was at C-4 and C-6—C-10. Appreciable decarboxylation and consequent label scrambling had occurred with carboxy-labelled acetate since the Ar–C_4 fragment contained a high fraction of the isotope although the major activity was at C-5 as expected. C-4—C-10 are thus of acetate–malonate origin. Their mode of assembly is indicated by the incorporation of sodium [1-^{14}C]hexanoate. Although some labelling of the Ar–C_4 unit resulted, probably *via* decarboxylation and incorporation of CO_2 (*cf.* the very similar result with $Me^{14}CO_2Na$), the bulk of the label appears in the C-5—C-10 unit, with all the activity in this section located at C-5, *i.e.* no label scrambling with the part of the molecule believed to be derived from hexanoate.

Some guidelines to the biosynthesis of [6]-gingerol now appear from these results and are shown in Scheme 2. In essence, Phe is elaborated to ferulic acid through *p*-coumaric acid. Reduction to dihydroferulic acid ensues, followed by condensation with a malonate and an hexanoate residue (preformed from acetate–malonate), in an undetermined order, *via* the 'biological Claisen reaction.' Such reactions are well known, but their scope is now seen to include the present case in which an acetate–malonate condenses with two other acids neither of which is a second acetate–malonate. We know of no other demonstrated example of this type.‡ The product [6]-gingerdione (**7**) would then be reduced at C-5 to give natural [6]-gingerol (**1**). Direct production of the β-ketol from malonate condensations with dihydroferulate and hexanal was excluded by the non-incorporation of [1-^{14}C]hexanal.



SCHEME 2. Proposed biosynthetic route to [6]-gingerol.

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‡ For a discussion of related cases see ref. 3 and refs. cited there.

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