Studies in the Biosynthesis of [6]-Gingerol, Pungent Principle of Ginger (*Zingiber officinale*)

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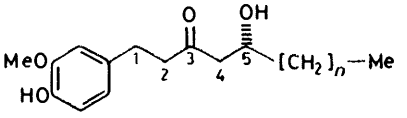
The biosynthesis of [6]-gingerol has been investigated by the administration of labelled precursors to whole *Zingiber officinale* plants and to rhizome sections. The results show that phenylalanine is elaborated to ferulic acid which then condenses, in an unusual version of the biological Claisen ' reaction, with malonate and hexanoate to yield [6]-dehydrogingerdione (11). Hexanoate is incorporated intact as shown by the location of label and the constancy of isotope ratios. Dihydroferulate is accepted, but loss of tritium shows it to be first dehydrogenated. Dione (11) is reduced in two steps to [6]-gingerol; in the preferred path, C=O reduction precedes C=C reduction, with (12) as intermediate, although dione (13) is also converted.

THE herbaceous perennial *Zingiber officinale* Roscoe, is grown commercially in most tropical regions for its rhizome, ginger, which is valued for its aroma and pungency. Ginger is mentioned in ancient Chinese, Hebrew, and Sanskrit literature, and the spice was being imported into Anglo-Saxon England in the ninth century.

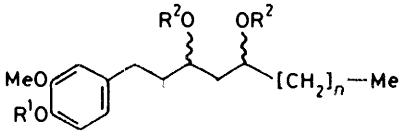
The first modern chemical investigations of the pungent constituents were made by Thresh [1] (1879), and the structure (1; $n = 4$, without stereochemistry) for the major phenol was demonstrated by Nomura [2] in 1928. However, the nature of the complex mixture of relatively unstable phenolic substances did not become clear until Connell and Sutherland [3] (1969) demonstrated the presence of an homologous series of phenolic ketones (1). The name [*m*]-gingerol for these compounds was proposed, where *m* = chain length of the aldehyde formed in retro-aldol cleavage; thus (1; $n = 4$) is [6]-gingerol (the major member of the series). The existence of [3], [4], [5], [6], [8], [10], and [12]-gingerols has been demonstrated: [3-5] ginger oleoresin also contains [4] the [6], [8], and [10]-gingerdiols (2; $n = 4, 6, 8$; $R^2 = H$), the diacetate (2; $n = 4$; $R^2 = Ac$), [6]-methylgingerdiol (3; $n = 4$, $R^2 = H$) and its diacetate (3; $n = 4$, $R^2 = Ac$). Hexahydrocurcumin [5] (4) is also present, forming an interesting biogenetic connection between ginger and turmeric (*Curcuma longa*) whose major pigment is curcumin (5). The enone (6), shogaol, is found in ginger oleoresin but not, to our knowledge, in fresh rhizome; it appears to be an artefact of extraction.[3] A related range of phenols, the paradols,[6] have been found in *Ammomum melegueta* (Grains of Paradise). In view of the apparent connections between the pathways involved in the biogenesis of the gingerols and curcumin, and of our previous work with turmeric pigments,[7] we have examined some aspects of the biosynthesis of [6]-gingerol and now report our results.†

RESULTS AND DISCUSSION

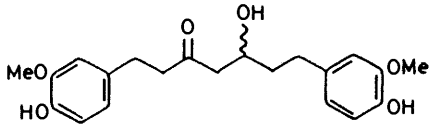
Methods.—Viable ginger rhizomes were obtained through the Tropical Products Institute,‡ grown in pots under glass. New rhizomes developed from swellings at the bases of shoots in late summer. Labelled precursors were administered by the wick method to stems just above new rhizome growth at this time. Simple



(1)



(2; $R^1 = H$)
(3; $R^1 = Me$)



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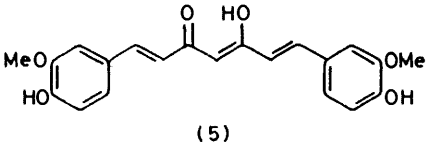

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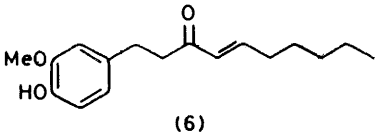


(6)

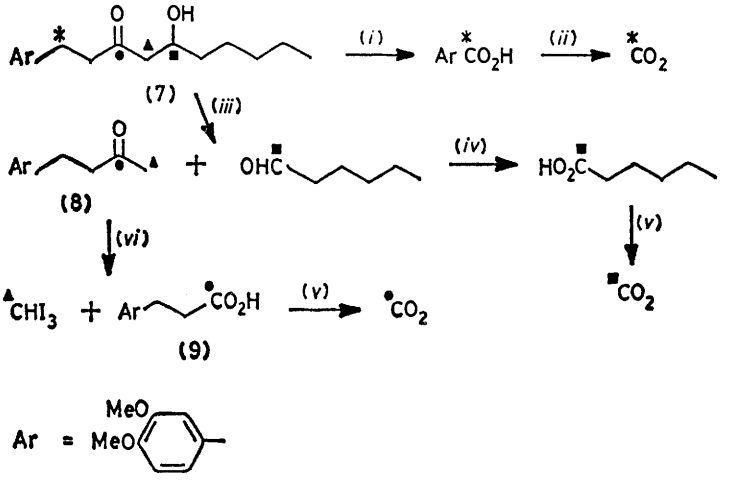
acids were applied as their sodium salts; phenols etc., of higher molecular weight were solubilised in methylcellosolve–water–Tween 20. The plants were grown on for six days after feeding and harvested. The rhizomes

† For preliminary communications see ref. 8.

‡ We are particularly grateful to Miss M. Burbage for her valuable assistance.

were cooled to −70 °C, crushed, and extracted with acetone to afford the crude oleoresin. Optimum yields of crystalline product were obtained by methylation of the total oleoresin, partition of the material between nitromethane and petrol, and chromatography of the polar fraction: *S*-methyl [6]-gingerol (7) was thus obtained, m.p. 64.5—65.5 °C, $[\alpha]_D^{23}$ −27.5: (EtOH) (*ca.* 10% yield from oleoresin). For location of labelled atoms the methyl ether (7) was degraded using the reactions of Scheme 1. Permanganate oxidation gave veratric acid, which was decarboxylated over copper to isolate C-1 as CO_2 (trapped as barium carbonate). The resulting [^{3}H]trifluoroacetic acid. Thus the content of ^{1}H in the hydrogen isotopes was minimised, consequently mitigating the unfavourable kinetic isotope effects on the introduction of ^{3}H in the catalytic hydrogenation.

[6-^{3}H]Hexanoic acid was formed in four stages from 6-chlorohexanol; replacement of chlorine by iodine, and trimethylsilylation proceeded essentially quantitatively, and the product (10) was converted into the corresponding Grignard reagent. Quenching with ^3H_2O gave [6-^{3}H]hexanol (80%), then further oxidised to [6-^{3}H]-hexanal and [6-^{3}H]hexanoic acid. The last two compounds were further employed (Scheme 2) in the synthesis of [10-^{3}H]dehydrogingerdione (11), (±)-[10-^{3}H]dehydrogingerol (12), and [10-^{3}H]gingerdione (13) by condensations [10] with the anions (14) and (15).



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

retro-aldol fission with alkali readily gave zingerone (8) but poor yields of hexanal: [3] however combined retro-aldol–oxidation using sodium hydroxide–silver oxide (generated *in situ*) afforded hexanoic acid in satisfactory yield. Zingerone (8) was cleaved to iodoform (C-4), and acid (9): decarboxylation of this acid and hexanoic acid was accomplished with lead tetra-acetate, thus separating C-3 and C-5.

Radio-labelled Precursors.—(±)-[1-^{14}C]- and L-[U-^{14}C]-phenylalanine, sodium [1-^{14}C]- and [2-^{14}C]- acetate, sodium [2-^{14}C]malonate, and [1-^{14}C]hexanoate were obtained from Amersham. [1-^{14}C]Hexanal was prepared from the corresponding acid by reduction with lithium aluminium hydride–lithium chloride and Cr^{VI} oxidation of the resulting hexanol. [Ar-^{3}H]-*p*-Coumaric acid and [Ar-^{3}H]ferulic acids were available from previous work,[7] and were hydrogenated to provide the corresponding dihydro-acids. [2-^{14}C]Ferulic acid was prepared by Doebner condensation of vanillin with [2-^{14}C]malonic acid, and hydrogenated to provide [2-^{14}C]-dihydroferulic acid. To prepare [2,3-$^3H_2$]dihydroferulic acid by [^{3}H]-hydrogenation, a Brown apparatus was employed, modified to permit evacuation,[9] in which tritiated deuterium was generated by (*i*) adding tritiated water to diglyme containing trifluoroacetic anhydride, and (*ii*) decomposing sodium borodeuteride with the

Results.—The various feeding experiments and their results are listed in Tables 1—5. The experiments of Tables 1, 2 and of Tables 3, 4 were carried out in different years; the second set were (unavoidably) performed with a weak, slow-growing, batch of plants, and dilutions were markedly higher with this group.* There are also differences between individual plants within a batch, which adds to the difficulties of interpretation of results. Table 5 contains the data obtained from incubating aqueous solutions of precursors with thin slices of rhizome (excised during active growth) for 24 h.

L-[U-^{14}C]- and (±)-[1-^{14}C]-Phenylalanine are both incorporated into [6]-gingerol at levels considered significant in experiments with whole plants, and degradations show the label(s) to be contained within the Ar-C_3 section of the molecule. [^{3}H]-*p*-Coumaric acid (16) and [^{3}H]ferulic acid (17) were both better precursors than Phe. The relatively low dilution of [^{3}H]-(17) may reflect the experimental difference that

* Because of increased difficulties in product purification, total incorporations could be calculated only approximately: hence Tables 4 and 5 show only specific incorporations.

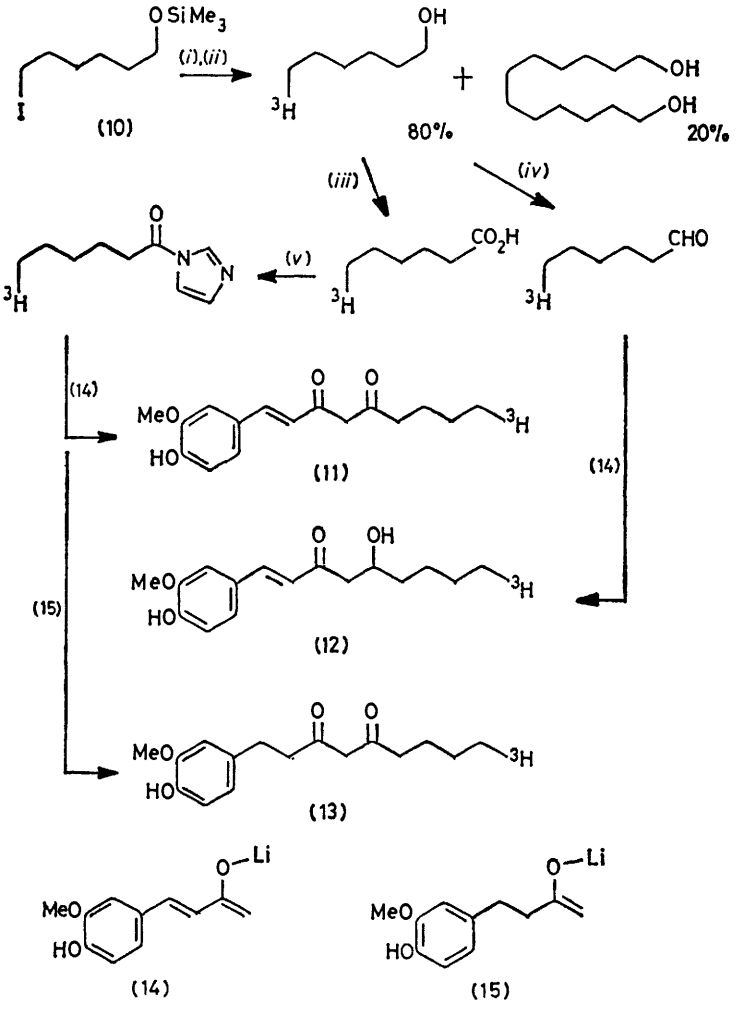
TABLE 1

Administration (6 d) of labelled precursors to whole *Z. officinale* (Season 1)

Expt. No.	Precursor specific activity (μCi mol^{-1})	Specific activity of [6]-gingerol (μCi mol^{-1})	Total incorporation (%)	Dilution
1	(±)-[1-^{14}C]-Phe (60)	5.14×10^{-3}	0.009	1.17×10^{7}
2	L-[U-^{14}C]-Phe (477)	3.36×10^{-3}	0.006	1.42×10^{8}
3	[2-^{14}C]-$MeCO_2Na$ (60)	3.24×10^{-2}	0.011	1.85×10^{6}
4	[1-^{14}C]-$MeCO_2Na$ (58)	5.81×10^{-3}	0.002	9.98×10^{6}
5	[1-^{14}C]-n-$C_5H_{11}CO_2Na$ (23.6)	1.06×10^{-2}	0.017	2.22×10^{-6}
6	[1-^{14}C]-n-$C_5H_{11}CHO$(5.27×10^{-3})	$<2.0 \times 10^{-6}$	$<10^{-7}$	

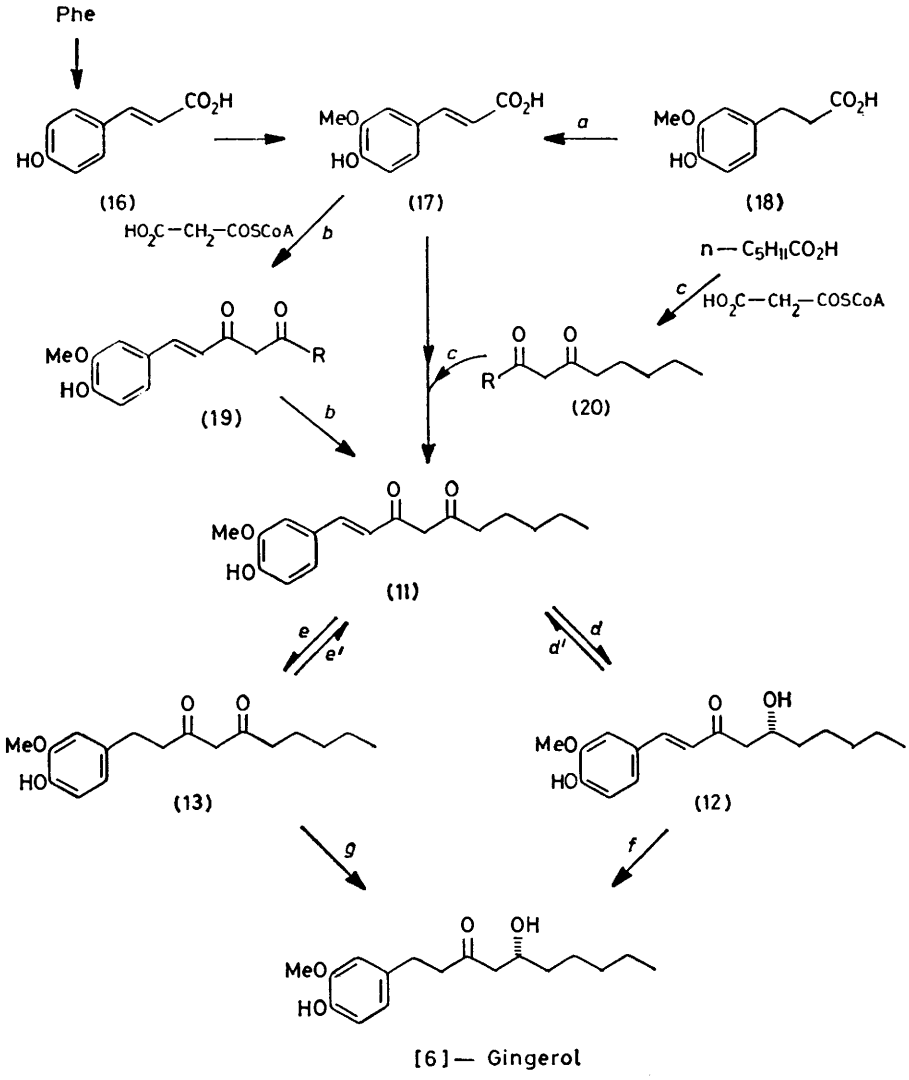
Expt. No.	Fractional distribution of label			
	Ar·$^{1}CH_2$·$^{2}CH_2$·^{3}CO	$^{4}CH_2$	$^{5}CH(OH)$	$^{6-10}C_5H_{11}$
1	0.89	0	0	0
2	0.99	0	0	0
3	0.05	0.49	0.03	0.43
4	0.29		0.46	0.26
5	0.30		0.70	0

only developing rhizomes were harvested; total rhizomes were collected in all other cases. [Ar-^{3}H]Dihydro-*p*-coumaric acid was a poor precursor but [Ar-^{3}H]dihydroferulic was utilised at a significant level, with an incorporation of the same order of magnitude as [Ar-^{3}H]-*p*-coumaric acid but with a substantially lower dilution. However, a further experiment indicates that dihydroferulic acid is not an obligate precursor to [6]-gingerol. [2-^{14}C, 2,3-^{3}H]Dihydroferulic acid was also applied to ginger plants, and the ^{3}H : ^{14}C ratios in precursor and



SCHEME 2 Synthesis of ω-^{3}H precursors (*i*) Mg–Et_2O; (*ii*) ^3H_2O, H^+; (*iii*) $KMnO_4$, H^+, Me_2CO; (*iv*) CrO_3, pyridine; (*v*) bis(imidazolyl) ketone

product measured (Table 3). Despite the rather high dilutions (attributed to poor plant growth in the second season) the results 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 labelled acetate, appreciable label-scrambling was observed since the Ar-C_4 fragment contained a high fraction of the activity, although the major quantity of isotope was located at C-5. Such scrambling is frequently observed with [1-^{14}C]acetate and is ascribed to decarboxylation. These two experiments indicate that the seven-carbon unit, C-4—C-10, is of acetate–malonate



SCHEME 3 Proposed biosynthesis of [6]-gingerol

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.

Both sodium [1-^{14}C]- and [2-^{14}C]-acetate were incorporated into [6]-gingerol. With methyl-labelled acetate very little radioactivity was found, on degradation, in the Ar-C_3 unit or at C-5; the major fraction was located at C-4 and the remainder at C-6—C-10. With carboxy-origin. Various modes of assembly may be envisaged: however three experiments indicate rather clearly that the biosynthesis involves an intact unit of hexanoic acid (formed of course from three acetate–malonates). First, sodium [1-^{14}C]hexanoate is incorporated into whole plants, at approximately the same level of incorporation as [2-^{14}C]acetate. On degradation it was seen that the major part of the label appeared in the C-5—C-10 unit with all the activity in this section localised at C-5; *i.e.* there is no label-scrambling within this section. Some labelling of the Ar-C_4 part resulted probably *via* decarboxylation and incorporation of

* An *o*-coumarate reductase has been described, with a low activity towards ferulate (C. C. Levy and G. D. Weinstein, *Biochemistry*, 1964, **3**, 1944).

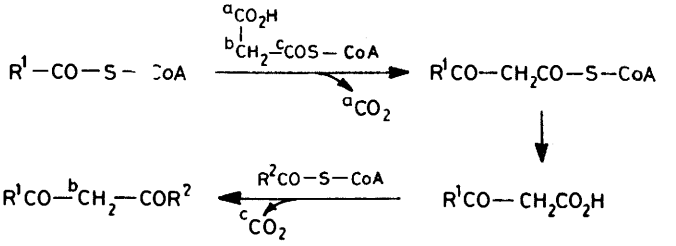
TABLE 2

Administration (6 d) of labelled precursors to whole *Z. officinale* (Season 1)

Precursor (specific activity) (disint. min^{-1} $mmol^{-1}$)	Specific activity of [6]-gingerol, (disint. min^{-1} $mmol^{-1}$)	Total incorporation (%)	Dilution
[^{3}H]Dihydro-*p*-coumaric acid (8.92×10^{10})	1.26×10^6	0.004	7.08×10^4
[^{3}H]-*p*-Coumaric acid (8.92×10^{10})	3.65×10^6	0.62	2.44×10^4
[^{3}H]Ferulic acid (7.50×10^7)	1.26×10^6	0.249 *	59.5
[^{3}H]Dihydroferulic acid (7.50×10^7)	1.09×10^5	0.033	6.88×10^2

* Only developing rhizomes harvested.

CO_2 (*cf.* the very similar result with $Me^{14}CO_2Na$). Secondly, in the experiments (Table 5) with sliced rhizomes it was noted that the dilution of hexanoate was 330-fold lower than that of acetate. Finally, the possibility that label from hexanoate was being built into [6]-gingerol by way of catabolism to acetate was denied by the experiment with [1-^{14}C,6-^{3}H]hexanoate (Table 3). The $^3H : {}^{14}C$ ratio is virtually unchanged between precursor and metabolite, showing that the complete C_6 molecule has been utilised. Only a little (*ca.* 3%, from the change in $^3H : {}^{14}C$ ratio) decarboxylation was observed in this experiment. Thus the origins of Ar-C-1—C3 and C-5—C-10 are accounted for; C-4 is supplied by $^{14}MeCO_2Na$ and must be considered to be derived from malonate. Scheme 3 indicates the biosynthetic routes consistent with this evidence. Phenylalanine is elaborated to ferulic acid (17) through *p*-coumaric acid (16). Condensation of acid (17) with malonate and hexanoate must then ensue, either by path *b* [ferulate reacts with malonyl CoA to form β-ketoester (19; R = S-CoA): hydrolysis to (19; R = OH) must follow, and condensation with hexanoyl-CoA], or by path *c* [hexanoate reacts first with malonyl-CoA, to yield (20; R = S-CoA); after hydrolysis (20; R = OH) then condenses with feruloyl-CoA]. The order of the two C–C bond-forming steps (shown in general form in Scheme 4) cannot be determined from the present evidence. The type of ' biological Claisen ' reaction shown in Scheme 4, in which the malonate loses *both* carboxys in sequence, is novel. Such a scheme was postulated for curcumin biosynthesis some time ago,[7] but could not be experimentally verified.[11] The only other case known to us is that involved in the biosynthesis of 9-phenylphenalenones.[12]



SCHEME 4

Both pathways *b* and *c* lead to [6]-dehydrogingerdione (11) as the first intermediate with the complete C-skeleton of [6]-gingerol. Direct production of a β-ketol, *i.e.* (12), by condensations involving hexanal was excluded by the non-incorporation of [1-^{14}C]hexanal (Table 1). Two reduction steps are then necessary to transform (11) to (1; $n = 4$), and either [6]-gingerdione (13) or [6]-dehydrogingerol (12) must be an intermediate. Examination of Scheme 3 suggests close parallels between certain pairs of steps, *i.e.* the two carbonyl reductions *d* and *g*; the two C=C reductions *e* and *f*; and the dehydrogenations *a* and *e'*. Such similar pairs may be mediated by the same, not completely substrate-specific enzyme system. It was not therefore unexpected to find (Table 4) that all three compounds (11), (12), and

TABLE 3

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

Precursor	Dilution (^{14}C)	(A) $^3H/^{14}C$ (Precursor)	(B) $^3H/^{14}C$ (6-Gingerol)	B/A
[2-^{14}C, ^{3}H, 3-^{3}H]Dihydroferulate	8.4×10^4	18.8	9.9	0.53
[1-^{14}C, 6-^{3}H]Hexanoate	5.8×10^6	6.6	6.8	1.03

TABLE 4

Administration of ^{3}H-precursors to *Z. officinale*

Precursor	Precursor specific activity (μCi $mmol^{-1}$)	Me[6]-Gingerol specific activity (μC, $mmol^{-1}$)	Specific incorporation (%)	Dilution
[10-^{3}H]Dehydrogingerdione	110	0.01	0.009	1.1×10^4
[10-^{3}H]Dehydrogingerol *	626	0.36	0.12	8.5×10^2
[10-^{3}H]Gingerdione	232	0.009	0.004	2.6×10^4

* Corrected for incorporation of a single enantiomer.

(13) were all fairly well incorporated into [6]-gingerol. However, the ketol (12) was markedly less diluted than the obligatory dehydrodione (11), while the highest dilution was observed for the dione (13).

On the surface, these data indicate that the preferred route from (11) to [6]-gingerol is *df*, *via* (12), while (13) is converted inefficiently, either by way of a direct but slow reduction step *g*, or indirectly through the *e'df* sequence. Further double labelling experiments with (12) and (13) may further illuminate the workings of this grid. In one isolated experiment (13) was incubated with rhizome sections (Table 5), and converted with a very low dilution into [6]-gingerol; this type of experiment promises to be of value in further work.

TABLE 5

Administration (24 h) of labelled precursors to rhizome sections (Season 2)

Precursor specific activity, (μCi mmol^{-1})	Specific activity of [6]-gingerol (μCi mmol^{-1})	Specific incorporation (%)	Dilution
[2-^{14}C]-$MeCO_2Na$ (58×10^3)	0.30	0.0005	1.9×10^5
[6-^{3}H]-n-C_5H_{11}·CO_2Na (4.934×10^3)	8.46	0.17	573
[10-^{3}H]-[6]-Gingerdione (2.317×10^2)	4.77	2.06	48.6

EXPERIMENTAL

Thin layer and thick layer (preparative) chromatography was performed with HF_{254} silica (Merck). Radioactivity was measured by liquid-scintillation methods using a Nuclear Enterprises N.E. 8310 automatic spectrometer. Dioxan-based (for polar compounds) and toluene-based scintillators (NE 250 and NE 233 respectively) were employed. Counting efficiencies were determined either by internal standard or by calibrated external standard. Barium carbonate was counted as a suspension in a gel, using Cab-O-Sil gelling agent.

Growth and Feeding of Z. officinale.—Viable ginger rhizomes were supplied, through the agency of the Tropical Products Institute, from St. Lucia, and grown individually in 18-cm pots at 35 °C. After 3—4 months mature plants (*ca.* 50 cm tall) developed basal swellings of the stems. Aqueous solutions of precursors were wick-fed to stems during the expansion of new rhizomes, over 6 days. Acidic compounds were dissolved in water (0.5—3 cm^3) containing 1 mol equiv. of sodium hydroxide. Neutral compounds were dissolved in the minimum methylcellosolve and diluted with water (6 cm^3) containing sufficient Tween-20 to produce a clear 'solution' For the experiments with rhizome sections, newly formed rhizomes were used, cut into slices *ca.* 2-mm thick, and incubated at 35 °C in the light for 24 h with aqueous solutions of precursors.

Extraction of Ginger Rhizomes and Isolation of Methyl [6]-*Gingerol*.—Fresh ginger rhizomes (100 g) were frozen in liquid nitrogen and powdered. The powder was extracted at ambient temperature with acetone (300 cm^3) overnight and twice with refluxing acetone (300 cm^3). The combined solutions were set aside at −30 °C for 48 h, and precipitated waxes filtered off. The solution was concentrated, diluted with water (100 cm^3), and the pH adjusted to 7.0 with sodium hydrogencarbonate. The neutral solution was then extracted with ether (3×), and the extracts washed, dried, and evaporated to yield the dark brown, pungent, oleoresin (*ca.* 600 mg; 0.6%). This oil was then dissolved in 2M sodium hydroxide (13 cm^3); methyl sulphate (1.06 g) was added dropwise, and the mixture shaken for 1 h, when it was acidified (concentrated hydrochloric acid) and extracted with ether. The extracts were washed, dried, and evaporated. The residue was partitioned between nitromethane (20 cm^3) and light petroleum (2 × 15 cm^3), b.p. 60—80 °C. The product was isolated from the nitromethane phase by dilution with water and extraction into ether: it was purified by p.l.c. using hexane–ether (9 : 1). The major band yielded methyl [6]-gingerol, (80 mg; 0.08% from rhizomes), m.p. 64.5—65.5 °C (lit.,[3] m.p. 65—66 °C), $[\alpha]_D^{25}$ +27.5° (EtOH), (lit.,[3] $[\alpha]_D^{23}$ +28.4°) (Found: C, 69.8; H, 9.4. Calc. for $C_{18}H_{28}O_4$, C, 70.1, H. 9.1%); $\nu_{max.}$ (KBr) 1 703 cm^{-1}; $\lambda_{max.}$ (EtOH) 229 (3.86), 279 nm (3.42); τ($CDCl_3$) 3.25 (3 H, m, Ar-H), 5.96 (1 H, m, C*H*OH), 6.11 (3 H, s, OMe), 6.13 (3 H, s, OMe), 7.19 (4 H, m, AeCH_2CH_2CO), 7.47 (2 H, d, *J* 6 Hz, COCH_2CHOH), 8.7 (8 H, m, [CH_2]$_4$), and 9.11 (3 H, t, *Me*); *m/e* 308.197 (calc. for $C_{18}H_{28}O_4$, 308.197).

Oxidation of Methyl [6]-*Gingerol*.—Methyl gingerol (170 mg) was mixed with 2M aqueous sodium carbonate (10 cm^3) and the minimum dioxan required to effect solution. The solution was heated to reflux and 3% aqueous potassium permanganate added dropwise until the pink colouration persisted for 15 min. The mixture was acidified with concentrated hydrochloric acid and manganese dioxide dissolved by passing in sulphur dioxide. Veratric acid (70 mg, 70%) was isolated by extraction into ethyl acetate (3 × 10 cm^3), and sublimed at 125 °C and 0.01 mm: it had i.r., u.v., and n.m.r. spectra identical with an authentic sample.

Retro-Aldol Cleavage of Methyl [6]-*Gingerol under Oxidising Conditions*.—Silver nitrate (82.4 mg) in water (2 cm^3) was added to methyl gingerol (54.2 mg) in ethanol (5 cm^3). Sodium hydroxide (50 mg) in water (0.5 cm^3) was added with stirring and the mixture heated to reflux. After 15 min, more sodium hydroxide (50 mg) in water (0.5 cm^3) was added, followed by a third portion (100 mg) in water (1 cm^3) after a further 15 min. Refluxing was continued for 1 h when the mixture was filtered, and the solids washed with water and chloroform. The filtrate was extracted with chloroform (3 × 10 cm^3) and the combined organic phases evaporated to yield methylzingerone (8), (25.8 mg, 71%), m.p. and mixed m.p. with a synthetic sample.

The aqueous phase was acidified and crude hexanoic acid (26.5 mg) was collected through chloroform extraction: the *p*-bromobenzylpseudothiouronium salt was formed in the usual manner (16.5 mg, 46%) from aqueous ethanol, m.p. 145 °C (lit.,[13] m.p. 145 °C), undepressed by admixture with an authentic specimen.

Iodoform Reaction with Methylzingerone.—Methyl-

zingerone (9.7 mg) was dissolved in water (2 cm^3) containing the minimum dioxan, and 8% aqueous sodium hydroxide (2 cm^3) added. An aqueous solution of iodine (10%) and potassium iodide (20%) was added dropwise to the mixture with stirring, until the brown colouration persisted for 20 min. An equal volume of water was added and the mixture cooled; iodoform (4.2 mg, 23%) crystallised out, and was recrystallised from aqueous acetone. The aqueous phase was acidified, treated with sulphur dioxide, and extracted with chloroform. Organic acids were then washed into aqueous sodium hydrogencarbonate and collected into chloroform after acidification. P.l.c. (chloroform–ether, 9 : 1) yielded 3-(3,4-dimethoxyphenyl)-propionic acid (5.0 mg, 51%), sublimed at 85 °C and 0.01 mmHg, and identified by spectroscopic comparison with an authentic specimen.

Decarboxylations.—(*a*) The acid (0.55 mmol) was heated at 230 °C in quinoline (5 cm^3) containing copper powder (200 mg) for 2 h, under carbon dioxide-free nitrogen. Carbon dioxide was trapped with saturated aqueous barium hydroxide and the precipitated barium carbonate collected by filtration. In this way veratric acid gave barium carbonate (58 mg, 50%). (*b*) The acid (0.86 mmol) with lead tetra-acetate (90 mg) in benzene (25 cm^3) was stirred under a carbon dioxide-free atmosphere until an orange solution was obtained. Anhydrous lithium chloride (800 mg) was then added, and the mixture refluxed for 3 h; carbon dioxide was collected as barium carbonate. In this way veratric acid gave barium carbonate (77 mg, 46%); 3-(3,4-dimethoxyphenyl)propionic acid gave barium carbonate (109.5 mg, 65%), and hexanoic acid gave barium carbonate (135 mg, 80%).

[Ar-^{3}H]*Dihydro*-p-*coumaric Acid.*—[Ar-^{3}H]Coumaric acid (6.9 mg, 50 mCi $mmol^{-1}$) in methanol (2 cm^3) containing 2M hydrochloric acid (25 mm^3) was stirred under hydrogen, over platinum black, for 3 h. Filtration and evaporation afforded the title acid (6.9 mg), m.p. 128 °C.

[Ar-^{3}H]*Dihydroferulic Acid.*—[Ar-^{3}H]Ferulic acid (12.6 mg, 35 mCi $mmol^{-1}$) was hydrogenated as in the previous experiment. The product was purified by t.l.c. (benzene–dioxan–acetic acid, 18 : 5 : 0 : 5) to yield the title acid (7.9 mg), m.p. 89.5—91 °C.

[2-^{14}C]*Dihydroferulic Acid.*—[2-^{14}C]Malonic acid (2.174 mg, 17mCi $mmol^{-1}$), malonic acid (23.98 mg), vanillin (76.44 mg), pyridine (200 mm^3), and aniline (7.6 mm^3) were heated together at 55 °C overnight. The mixture was then shaken with ice (1 g) and concentrated hydrochloric acid (0.5 cm^3). After neutralisation with sodium hydrogencarbonate the mixture was washed with ether (2 × 10 cm^3), acidified, and the organic acids collected in ether (5 × 10 cm^3). After isolation in the usual way the crude ferulic acid was hydrogenated (Brown apparatus) in ethyl acetate over platinum black. Removal of the catalyst and evaporation of the solvent provided the title acid, recrystallised from ethyl acetate–light petroleum to constant m.p. and specific activity (10.5 mg), m.p. 89.5—91 °C, 673.2 μCi $mmol^{-1}$.

[2,3-$^3H_2$]*Dihydroferulic Acid.*—Trifluoroacetic anhydride (100 mg) in dry diglyme (1 cm^3) was added to tritiated water (40 mm^3, 90 mCi $mmol^{-1}$) in dry diglyme (1 cm^3), in the generator bulb of a Brown micro-hydrogenator (modified [9] to permit evacuation). To this was added sodium borodeuteride (20 mg) in diglyme (1 cm^3) and dimethylformamide (1 cm^3), generating a mixture of hydrogen isotopes. Ferulic acid (20 mg) in ethyl acetate (12 cm^3) was hydrogenated over platinum black. Isolation of the product through filtration and evaporation gave [2,3-$^3H_2$]dihydroferulic acid (9.8 mg) after crystallisation from ethyl acetate–light petroleum to constant m.p., 89.5—91 °C, and activity (5.518 mCi $mmol^{-1}$).

[1-^{14}C]*Hexanal.*—Sodium[1-^{14}C]hexanoate (50 μCi) was diluted with unlabelled salt (52.8 mg) and stirred with anhydrous lithium chloride (49 mg) in dry tetrahydrofuran (15 cm^3). Lithium aluminium hydride (17.4 mg) was added with stirring during 30 min. The mixture was refluxed for 15 min, cooled, and diluted with water and dilute sulphuric acid. Crude hexanol (84%) was extracted into chloroform (3 × 10 cm^3), washed with aqueous sodium hydrogencarbonate and recovered by evaporation. G.l.c. of the product showed a single peak inseparable from authentic hexan-1-ol. The alcohol was oxidised (without further purification) in dry dichloromethane with the dipyridine–chromium trioxide complex [from chromium trioxide (1.2 g) and pyridine (1.9 g) in dichloromethane (15 cm), stirred together for 1 h] at ambient temperature for 15 min. The solution was decanted and the solids washed with ether. After washing the bulked organic phases with 2N sodium hydroxide, 2N hydrochloric acid, aqueous sodium hydrogencarbonate, and water, they were dried and evaporated to yield [1-^{14}C]hexanal (28.5 mg. 70%, 52.7 Ci $mmol^{-1}$). The product was analysed by linked g.l.c.-scintillation counter and showed single coincident peaks for material and radioactivity.

6-*Iodo*-1-(*trimethylsilyloxy*)*hexane.*—6-Chlorohexan-1-ol (5 g) and sodium iodide (6 g) were allowed to react in acetone for 48 h at room temperature and 5 h at reflux. After filtration the solvent was evaporated and the residue extracted with ether. The extracts were washed with aqueous sodium thiosulphate, dried, and evaporated to yield 6-iodohexan-1-ol (8.85 g, 97%) [*m/e* 228 (M^+)]. The alcohol (4.56 g), hexamethyldisilazane (3.6 g) and trimethylsilyl chloride (0.2 cm^3) were refluxed together in dry benzene (15 cm^3) for 3 h, when sublimation of ammonium chloride was complete. The solution was evaporated and the residue distilled, to afford 6-*iodo*-1-(*trimethylsilyloxy*)-*hexane*, (4.38 g, 73%), b.p. 122 °C at 3.75 mmHg (Found: C, 36.35; H, 6.9%; *M*, 300.044. $C_9H_{21}IOSi$ requires C, 36.0; H, 7.0%; *M*, 300.041); $\nu_{max.}$ (liq.) 1 250, 1 100, and 835 cm^{-1}; τ($CDCl_3$) 6.38 [2 H, t, *J* 7 Hz, CH_2(OTMS)], 6.79 (2 H, t, *J* 7 Hz, CH_2I), 8.58 (8 H, m, $[CH_2]_4$), and 9.84 (9 H, s, $SiMe_3$).

[6-^{3}H]*Hexanol and* [6-^{3}H]*Hexanoic Acid.*—6-Iodo-1-(trimethylsilyloxy)hexane (1 g) was added dropwise to magnesium (0.12 g) under dry ether, with stirring: when reaction was complete tritiated water (20 mm^3; 90 mCi $mmol^{-1}$) was added, with stirring, followed by an excess of water. The organic products were collected through ether extraction, and refluxed in ethanol (40 cm^3) with 2M sulphuric acid (3 cm^3) for 1 h. The mixture was neutralised (potassium carbonate), the ethanol was evaporated, and the residue extracted with chloroform. The extracts, after drying and evaporation, gave [6-^{3}H]hexanol, which was diluted with unlabelled hexanol and distilled (bulb-to-bulb apparatus). The [6-^{3}H]hexanol (311 mg) in acetone (10 cm^3) was added to a stirred solution of potassium permanganate (1.2 g) in water (30 cm^3) with 2M sulphuric acid (7 cm^3). After 1 h the mixture was extracted into ether, which on evaporation and distillation (bulb-to-bulb) gave [6-^{3}H]hexanoic acid (237 mg, 67%; 4.93 mCi mm^{-1}).

[10-^{3}H]-[6]-*Dehydrogingerol.*—[6-^{3}H]Hexanol was oxid

ised to [6-^{3}H]hexanal, as described above, and allowed to react with lithiated trimethylsilyl vanillacetone, as described elsewhere.* The title compound was recrystallised to constant m.p., 134—137 °C, and activity, 626 μCi mmol^{-1}.

[10-^{3}H]-[6]-*Gingerdione*.—[6-^{3}H]Hexanoic acid was converted into [6-^{3}H]-hexanoylimidazole which was treated with lithiated trimethylsilylzingerone as described elsewhere.* The title compound, an oil, was purified by repeated p.l.c. to activity 231.7 μCi mmol^{-1}.

[10-^{3}H]-[6]-*Dehydrogingerdione*.—[6-^{3}H]-Hexanoylimidazole was treated with lithiated trimethylsilyl vanillacetone, as described elsewhere.* The product was crystallised to constant m.p., 83.5—84.5 °C, and activity, 110 μCl mmol^{-1}.

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* The methods for these preparations have been described in a communication; [10] experimental details will be included in a full paper (in preparation) on synthesis of gingerol homologues and relatives.

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