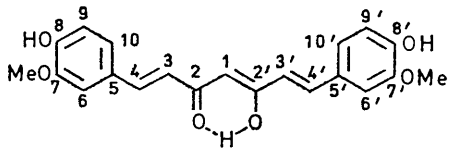
Experiments in the Biosynthesis of Curcumin

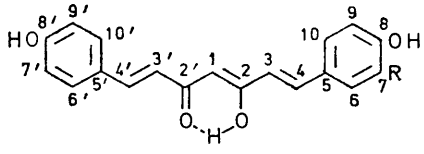
By **Peter J. Roughley** and **Donald A. Whiting,*** Department of Chemistry, The University, Nottingham NG7 2RD

The biogenesis of natural diarylheptanoids is discussed, with particular reference to curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione], the pigment of *Curcuma longa* rhizome. Methods for the isolation, characterisation, and degradation of curcumin, suitable for biosynthetic work, are reported. In administration of labelled precursors to *C. longa*, [1- and 3-^{14}C]phenylalanine were incorporated into curcumin without scrambling of the label. [1- and 2-^{14}C]-Acetate and -malonate were also incorporated, and the fractional distribution of label along the heptane chain was determined; the results do not provide satisfactory support for the expected biosynthetic scheme, in which two cinnamate units condense with one malonate unit. Other interpretations are discussed. [^{3}H]-4-Hydroxy-3-methoxy-, -4-hydroxy-, and -3,4-dihydroxy-cinnamic acids were prepared, and supplied to *C. longa* with [^{14}C]phenylalanine. The first two cinnamic acids are incorporated into curcumin significantly better than the last, although none was utilised quite as efficiently as phenylalanine.

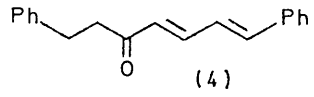
CURCUMIN (1), the yellow pigment of *Curcuma longa* (turmeric) and other *Curcuma* spp., has attracted few modern studies, although isolated as early as 1815.[1] It was crystallised by Daube[2] and the structure was elucidated in 1910 by Lampe and co-workers,[3] who later completed a synthesis.[4] Renewed interest has been evoked by the recent discovery of relatives sharing the 1,7-diaryl skeleton. These include 4-hydroxycinnamoyl-(4-hydroxy-3-methoxycinnamoyl)methane (2) and bis-(4-hydroxycinnamoyl)methane[5] (3) (both from *Curcuma*



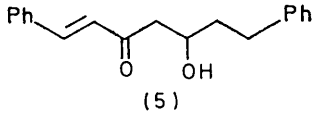
(1)



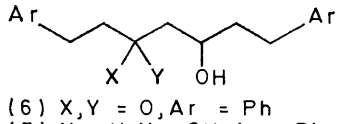
(2) R = OMe
(3) R = H



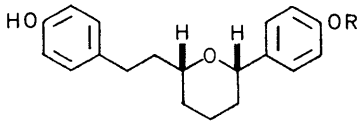
(4)



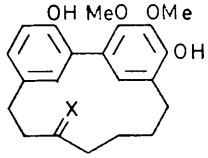
(5)



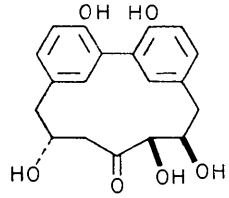
(6) X,Y = O, Ar = Ph
(7) X = H, Y = OH, Ar = Ph
(8) X = Y = H, Ar = *p*HO·C$_6$H$_4$



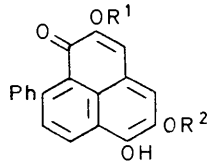
(9) R = Me
(10) R = H



(11) X = H, OH
(12) X = O



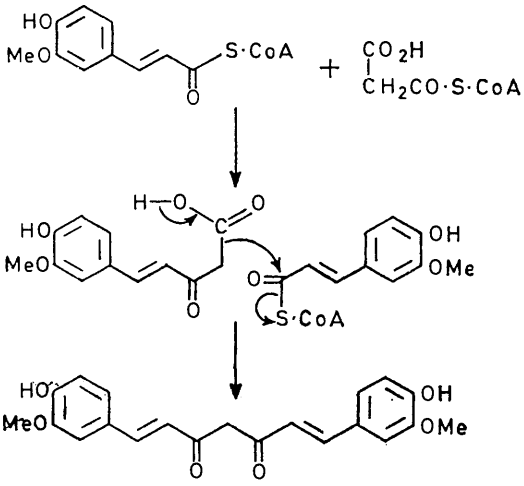
(13)



(14) R^1 = cellobiose, R^2 = Me
(15) R^1 = cellobiose, R^2 = H

[1] Vogel and Pelletier, *J. Pharm.*, 1815, **2**, 50.
[2] F. V. Daube, *Ber.*, 1870, **3**, 609.
[3] V. Lampe, J. Milobedzka, and St. v. Kostanecki, *Ber.*, 1910, **43**, 2163.
[4] V. Lampe and J. Milobedzka, *Ber.*, 1913, **46**, 2235.
[5] K. R. Srinivasan, *J. Pharm. Pharmacol.*, 1953, **5**, 448.

spp.), the extractives (4)—(7) [6] of male flowers (catkins) of certain *Alnus* spp., and centrolobol (8), centrolobin (9), and de-*O*-methylcentrolobin (10)[7] (*Centrolobium* spp.). The *m,m*-bridged biphenyls myricanol (11)



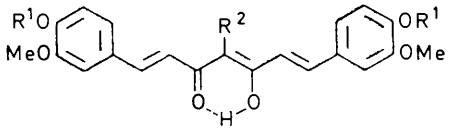
SCHEME 1

and myricanone (12) [8] (*Myrica nagi*), and asadanin (13) and its relatives [9] (*Ostrya japonica*) are also closely related in structure, as are the 9-phenylphenalen-1-ones haemocorin [10] (14), lachnanthoside (15), lachnanthofluorone, and lachnanthocarpone.[11]

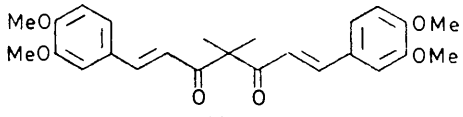
The simpler diarylheptanoids (1)—(10) show a pattern of 3(5) oxygenation (in the heptane chain), and 1,2 (6,7) unsaturation is common. More complex oxidation levels, including oxidative coupling, are found in the other examples (11)—(15). This pattern has provoked the very reasonable suggestion [12] that the biosynthesis of curcumin involves two cinnamate units which are coupled to a central carbon provided by malonate (Scheme 1), and it was in the expectation of supporting the outline of this scheme that, following structural work in the area,[8] we initiated the experiments in biosynthesis described in this paper.

Curcumin was chosen for biosynthetic study since it occurs in the rhizomes of the tropical perennial *Curcuma longa*,* which are suitable for *in vivo* experiments, unlike most other sources of diarylheptanoids. Prerequisite for such experiments are satisfactory methods for the isolation, purification, and characterisation of curcumin, and these are now discussed.

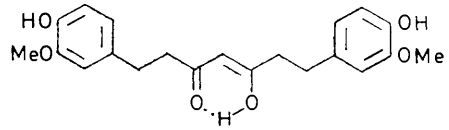
The curcuminoids (1)—(3) were extracted from dried rhizome by benzene extraction; purification *via* the lead salts [13] was not necessary, direct separation by p.l.c. being satisfactory. None of the *cis*-isomers previously reported [5] were detected in this or other [14] isolation procedures. Synthetic specimens of the symmetrical curcuminoids (1) and (3) were prepared by the method of Pabon,[15] which was extended to produce the unsymmetrical diketone (2). Spectra of these compounds (and the derivatives described below) are reported in the Experimental section. N.m.r. spectra indicate that all the diketones (1)—(3) exist (in chloroform solution) entirely in the enolic forms as shown; † interchange between equivalent enols is presumably rapid. The enolic protons of (1)—(3) can be observed in the n.m.r. spectrum in [$^2H_6$]acetone solutions at −90 °C, although they are not apparent at room temperature.



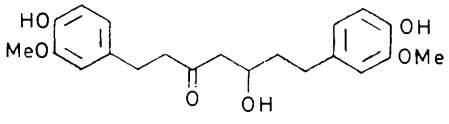
(16) R^1 = Me, R^2 = H
(18) R^1 = R^2 = Me
(19) R^1 = Ac, R^2 = H



(17)



(20)



(21)

Various derivatives of curcumin were investigated for characterisation purposes. Methylation with methyl

* We thank the Tropical Products Institute for viable *C. longa* obtained from India.

† Curcumin is sometimes drawn in this paper in the diketonic form to emphasise symmetry.

[6] (*a*) T. Suga, Y. Asakawa, and N. Iwata, *Chem. and Ind.*, 1971, 766; (*b*) Y. Asakawa, F. Genjida, S. Hayashi, and T. Matsuura, *Tetrahedron Letters*, 1969, 3235; (*c*) Y. Asakawa, *Bull. Chem. Soc. Japan*, 1970, **43**, 575, 2223; (*d*) N. I. Uvarova, G. I. Oshitok, A. K. Dzizenko, and G. B. Elyakov, *Khim. prirod. Soedinenii*, 1970, 463.

[7] (*a*) I. L. de Albuquerque, C. Galeffi, C. G. Casinovi, and G. B. Marini-Bettòlo, *Gazzetta*, 1964, **94**, 287; (*b*) A. Aragão Craveiro, A da Costa Prado, O. R. Gottlieb, and P. C. Welerson de Albuquerque, *Phytochemistry*, 1970, **9**, 1869.

[8] (*a*) R. V. M. Campbell, L. Crombie, B. Tuck, and D. A. Whiting, *Chem. Comm.*, 1970, 1206; (*b*) M. J. Begley and D. A. Whiting, *ibid.*, p. 1207; M. J. Begley, R. V. M. Campbell, L. Crombie, B. Tuck, and D. A. Whiting, *J. Chem. Soc.* (*C*), 1971, 3634.

[9] (*a*) M. Yasue, *J. Japan Wood Research Soc.*, 1965, **11**, 146, 153; (*b*) M. Yasue and H. Imamura, *ibid.*, 1966, **12**, 226, 231.

[10] (*a*) R. Thomas, *Biochem. J.*, 1961, **78**, 807; (*b*) *Chem. Comm.*, 1971, 739.

[11] (*a*) J. M. Edwards and U. Weiss, *Tetrahedron Letters*, 1969, 4325; (*b*) J. M. Edwards, R. C. Schmitt, and U. Weiss, *Phytochemistry*, 1972, **11**, 1717.

[12] *E.g.* T. A. Geissman and D. H. G. Crout, ' Organic Chemistry of Secondary Plant Products,' Freeman Cooper, San Francisco, 1969.

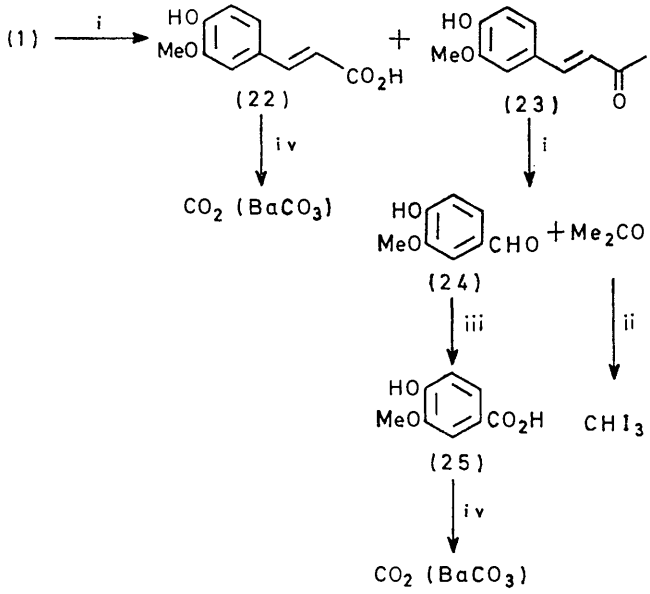
[13] A. D. Gol'tman, *Ukrain. khim. Zhur.*, 1957, **23**, 659.

[14] M. Kuroyanagi and S. Natori, *Yakugaku Zasshi*, 1970, **90**, 1467.

[15] H. J. J. Pabon, *Rec. Trav. chim.*, 1964, **83**, 379.

iodide is reported [16] to provide di-*O*-methylcurcumin (16), m.p. 135°. In our hands curcumin, when treated with methyl iodide in refluxing acetone, gave mainly the tetramethyl compound (17) m.p. 138°, although some trimethyl derivative (18), m.p. 152° was also obtained. A genuine dimethyl ether (16), m.p. 130°, was isolated from the products obtained on treating curcumin with dimethyl sulphate in benzene. Diacetyl curcumin [16a] (19) was readily prepared, but the ' diacetylisocurcumin ' of Ghosh [17] could not be obtained. The hydrogenation of curcumin was investigated with a variety of conditions and catalysts; however mixtures of tetrahydro- and hexahydro-curcumin (20) and (21) were always obtained, even using elevated temperature and pressure.

The question of specific atom or group isolation from curcumin, for assessment of tracer distribution, was then examined. The oxidation of curcumin to vanillin could be achieved with ozone or osmium tetroxide; however, the yields of aldehyde were low, and other fragments were not readily isolated. Veratraldehyde was obtained from tetramethylcurcumin more efficiently, also using osmium tetroxide. Alkaline degradation as shown in Scheme 2



SCHEME 2 Reagents: i, NaOH; ii, NaOH, KI, I_2; iii, Ag_2O, NaOH; iv, Cu, quinoline, Δ

was much more satisfactory. Cleavage of the β-diketone function gave ferulic (4-hydroxy-3-methoxycinnamic) acid (22) and feruloylmethane (23); the latter formed vanillin (24) and acetone by retro-aldol fission. The acetone was oxidised to iodoform and vanillin was oxidised to vanillic acid (25). Decarboxylation of vanillic and ferulic acids was effected by heating with copper powder in quinoline. In radioisotope experiments with *C. longa*, curcumin was isolated and after purification by p.l.c. to constant activity, it was degraded as in Scheme 2. Acetone was distilled from the reaction mixture and converted into iodoform which was recrystallised to constant activity. Vanillin and ferulic acid were isolated and purified by chromatography, and resublimed to constant activity; ferulic acid may be further purified through its *p*-bromo-*S*-benzylpseudothiouronium salt. Carbon dioxide from decarboxylation of ferulic and vanillic acids was trapped as barium carbonate; as a check on specific activity it was in some experiments liberated from barium carbonate and retrapped. All the experiments described in this paper were internally consistent, in that agreement (within experimental error) was obtained between the total activity of curcumin and the sum of activities of its degradation products. Radiochemical counting of all the products from curcumin, as just described, enables the fractional activity in individual atoms in the heptane chain, and the total in the aryl rings, to be calculated by simple sum and difference methods. Further degradation of the aromatic rings was not attempted in the present work. Since curcumin interchanges rapidly between two chemically-equivalent enolic forms (1) only the activity in the central carbon can be uniquely determined; the activity determined for other carbon positions is the mean of a pair of equivalent atoms. This limitation is important since it renders proof of an unsymmetrical mode of biosynthesis extremely difficult.

We now turn to labelled precursor incorporation experiments using *C. longa* plants. Tracers in aqueous solution were wick-fed to the stem of 3—4 month old plants, and feeding was timed to coincide with the time in which new rhizomes were being formed, *i.e.* when curcumin production was expected to be a maximum. The chosen precursors and the experimental results are presented in the Tables.

Tables 1A and 1B show the results from 6 day feeding experiments with phenylalanine, using both [1-] and [3-^{14}C] labelled precursor. Good incorporations (0·05—0·36%) were attained, and degradation shows specific site labelling of curcumin, as summarised in Table 1C. Biosynthesis thus appears to involve the phenylalanine–cinnamate pathway. The labelling pattern is consistent both with Scheme 1, and with other schemes discussed below. Tables 2A—C record the rather unexpected results obtained from feeding [1-] and [2-^{14}C]sodium acetate, and [1-] and [2-^{14}C]sodium malonate. If the simple Scheme 1 operates for the biosynthesis of curcumin, it would be predicted that [2-^{14}C]-acetate or -malonate precursor would provide the central carbon (C-1), with comparatively little activity in other parts of the molecule, while [1-^{14}C]-acetate or -malonate might be expected to be incorporated to a markedly smaller extent. The experiments show that although better incorporations were achieved in 6 day than in 2 day feeding times, incorporations of [1-^{14}C]-acetate and -malonate were as good as (or better than) those of the 2-labelled acids. The distribution of activity (Table 2C) shows that activity from C-2 of acetate or malonate is not confined

[16] (*a*) G. Ciamician and P. Silber, *Ber.*, 1897, **30**, 192; (*b*) C. L. Jackson and L. Clarke, *Amer. Chem. J.*, 1911, **45**, 48; 1908, **39**, 696.

[17] P. C. Ghosh, *J. Chem. Soc.*, 1919, 292.

TABLE 1A

Administration of [1- and 3-^{14}C]phenylalanine to *C. longa* (incorporation period 6 days)

Experiment no.	Precursor (activity/μCi)	Curcumin isolated (mg)	Curcumin added (mg)	Specific activity * (disint. min^{-1} mM^{-1})	Incorporation (%)	Dilution
1	[1-^{14}C]Phe † (100)	12·9	18·0	2·72 × 10^{6}	0·103	1·64 × 10^{4}
2	[1-^{14}C]Phe (50)	7·2	28·4	1·11 × 10^{6}	0·097	2·39 × 10^{4}
3	[3-^{14}C]Phe (100)	13·1	17·6	1·36 × 10^{6}	0·051	3·48 × 10^{4}
4	[3-^{14}C]Phe (50)	37·4	0	3·86 × 10^{6}	0·354	2·87 × 10^{4}

* Activity after dilution. † Phe = phenylalanine.

TABLE 1B

Degradation products from curcumin after feeding phenylalanine

Specific activity * (disint. min^{-1} mM^{-1})	Experiment no. 1	2	3	4
Iodoform	0	0	7·0 × 10^{3}	0
Vanillin	1·29 × 10^{4}	0·2 × 10^{5}	1·24 × 10^{6}	2·35 × 10^{6}
Ferulic acid	2·28 × 10^{6}	5·96 × 10^{5}	1·03 × 10^{6}	2·39 × 10^{6}

* Activity after dilution.

TABLE 1C

Fractional distribution of activity on feeding [1- and 3-^{14}C]Phenylalanine

Experiment no.	(ArCH═CH——CO)$_2$——CH$_2$	
1	0·003	0·997
2	0·03	0·97
3	0·99	
4	0·99	

TABLE 2A

Administration of [1-] and [2-^{14}C]sodium acetate and [1-] and [2-^{14}C]sodium malonate to *C. longa*

Experiment no.	Precursor	Curcumin isolated (mg)	Curcumin added (mg)	Specific activity [a] (disint. min^{-1} mM^{-1})	Incorporation (%)	Dilution
5	[2-^{14}C]Malonate [b]	22·5	17·0	6·54 × 10^{5}	0·032	3·58 × 10^{4}
6	[2-^{14}C]Malonate [b]	15·4	20·5	2·37 × 10^{6}	0·104	3·91 × 10^{3}
7	[2-^{14}C]Acetate [b]	14·0	18·5	3·22 × 10^{5}	0·013	1·63 × 10^{5}
8	[2-^{14}C]Acetate [b]	26·3	10·0	3·36 × 10^{5}	0·015	2·67 × 10^{5}
9	[1-^{14}C]Malonate [b]	26·4	10·9	8·20 × 10^{5}	0·038	1·26 × 10^{4}
10	[1-^{14}C]Acetate [b]	28·6	8·4	9·48 × 10^{5}	0·043	1·07 × 10^{5}
11	[2-^{14}C]Acetate [c]	22·1	9·9	1·62 × 10^{5}	0·006	5·31 × 10^{5}
12	[1-^{14}C]Acetate [c]	16·5	15·6	4·96 × 10^{5}	0·020	1·36 × 10^{5}

[a] Activity after dilution. [b] Incorporation period 6 days. [c] Incorporation period 2 days.

TABLE 2B

Degradation products from curcumin after feeding sodium acetate and sodium malonate

Specific activity * (disint. min^{-1} mM^{-1})	Experiment no. 5	6	7	8	9	10
Iodoform	1·82 × 10^{5}	9·33 × 10^{5}	5·78 × 10^{4}	2·33 × 10^{4}	0·33 × 10^{5}	0·39 × 10^{5}
Vanillin	1·29 × 10^{5}	2·95 × 10^{5}	1·03 × 10^{5}	4·77 × 10^{4}	3·20 × 10^{5}	3·69 × 10^{5}
Ferulic Acid	1·87 × 10^{5}	3·68 × 10^{5}	1·52 × 10^{5}	6·65 × 10^{4}	4·05 × 10^{5}	4·72 × 10^{5}
$BaCO_3$ (vanillic acid)	1·99 × 10^{4}	0·46 × 10^{5}	1·51 × 10^{4}	0·49 × 10^{4}	0·44 × 10^{5}	0·37 × 10^{5}
$BaCO_3$ (ferulic acid)	1·32 × 10^{4}	0·23 × 10^{5}	1·18 × 10^{4}	0·45 × 10^{4}	0·61 × 10^{5}	0·58 × 10^{5}

* Activity after dilution.

TABLE 2C

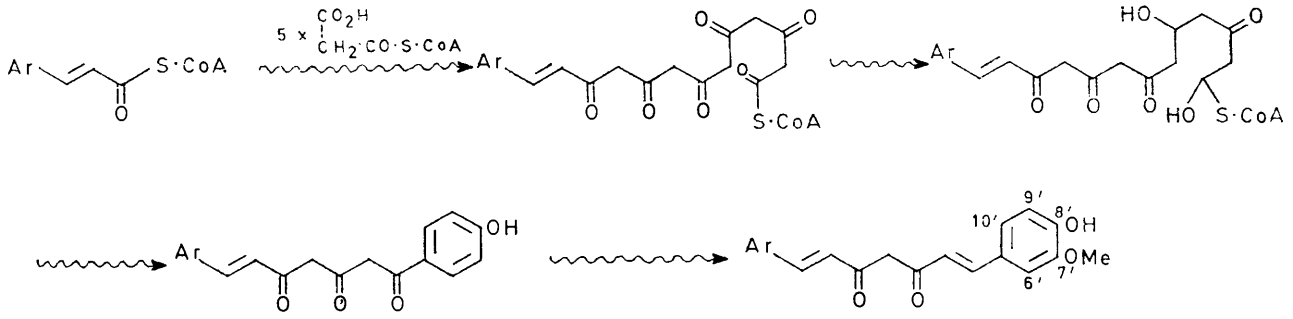
Fractional distribution of activity

Experiment no.	(Ar——CH═CH——CO)$_2$——CH$_2$ 4,4′	3,3′	2,2′	1	
5	0·31	0·06	0·13	0·04	0·46
6	0·20	0·03	0·04	0·02	0·71
7	0·46	0·08	0·19	0·06	0·21
8	0·52	0·06	0·17	0·05	0·20
9	0·65	0·10	0·06	0·14	0·05
10	0·68	0·08	0·09	0·12	0·03

to C-1 of curcumin, but labels other carbons in the heptane chain and the aryl rings. If biosynthesis does proceed by Scheme 1, some activity might be expected in the cinnamate-derived units, incorporated either *via* eventual participation of acetate, through pyruvate, in phenylalanine metabolism, or through degradation to carbon dioxide, but it would be surprising if such processes were more effective in acetate and malonate utilisation than the direct incorporation into the central methylene, unless some form of compartmentalisation operates.

will also differ in individual plants which unavoidably vary in growth rate and degree of development at the time of supplying potential precursors. The molar quantities of labelled compounds supplied were very much less than quantities of curcumin formed in the rhizome.

The general type of biosynthesis shown in Scheme 3 has been postulated for a wide range of natural compounds. Piperine [19] (*Piper nigrum*) may be derived from a cinnamic acid extended by one acetate or malonate



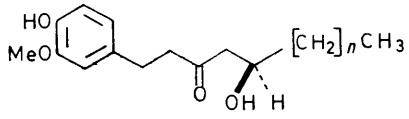
SCHEME 3

The fractional activities of Table 2C show, in general, an alternation in the level of activity of carbons along the heptane skeleton which is similar to that obtained in polyketide biosynthesis, and suggests a possible alternative route (Scheme 3) for curcumin formation. This could involve a cinnamate starter, extended by five acetate (malonate) units. Cyclisation of the chain gives the second aromatic ring (reduction before cyclisation removes the 6′- and 10′-hydroxy-functions), and biosynthesis would be completed by hydroxylation at C-7′. A number of variations of Scheme 3 are possible. The oxygenation pattern of the second aryl ring would then disguise its origin; however, further hydroxylation of acetate-derived rings is not unknown (*e.g.* pachyrrhizone [18]).

With, for example, [2-^{14}C]acetate (Experiments 7 and 8), label should be located at C-1 and C-3, with little activity at C-2 and C-4, if Scheme 3 operated without complication from compartmentalisation or the operation of other metabolic pathways. Some activity clearly is found at C-2 and C-4, which could have arisen by scrambling through CO_2 or (with [2-^{14}C]acetate) through the Krebs' cycle. Similar considerations apply to the other experiments in Table 2C.

Further inspection of Table 2C in connection with Scheme 3 reveals that the atoms in curcumin derived from, say, [2-^{14}C]-acetate or -malonate, are not equally labelled; this is not necessarily to be expected (although it is sometimes found experimentally) since malonate extension of a starter acid is evidently stepwise. The pool size of the various intermediates, and their rates of interconversion, will not be the same. These factors

unit; the addition of two such units may produce, *e.g.*, 2,6-dihydroxy-4-methoxybenzophenone [20] (*Nectandra coto*), while the pattern of extension by malonate units is well proven in the flavonoid, isoflavonoid, and stilbene groups. *trans*-Stilbene itself (without residual oxygens on the acetate-derived ring) occurs in *Alnus sieboldiana* together with diarylheptanoids. The gingerols [21] (26) (*Zingiber officinalis*) may have their origin in a cinnamate



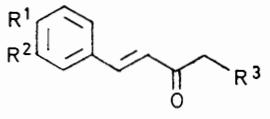
(26) n = 4, 6, or 8

unit condensed with 4—6 malonates, and it is worthy of note that hexahydrocurcumin (21) has very recently also been identified [22] as a natural constituent of *Z. officinalis*.

The effective symmetry of the tautomeric curcumin molecule and the degree of isotopic scrambling in the precursor experiments just described prevents a clearcut interpretation of those experiments in favour of either Scheme 1 or 3 for the biosynthesis. Neither Scheme should be accepted without new experimental evidence, which will probably require either work with unsymmetrical diarylheptanoids, or refinement of the biological system for the study of curcumin. One approach which we could not bring to fruition involved synthesis of β-ketoacids such as (30); the intact incorporation of such acids would require a biosynthetic route like that in Scheme 3. Although cinnamoylmethane (27) was successfully carboxylated to the unstable β-ketoacid (28),

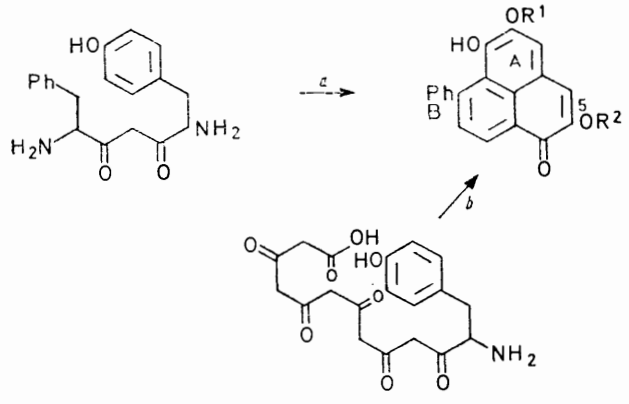
[18] H. Bickel and H. Schmid, *Helv. Chim. Acta*, 1953, **36**, 664.
[19] L. Rügheimer, *Ber.*, 1882, **15**, 1390.
[20] E. Spath and E. Wessely, *Monatsh.*, 1928, **49**, 229.
[21] D. W. Connell and M. D. Sutherland, *Austral. J. Chem.*, 1969, **22**, 1033.
[22] T. Murata, M. Shinohara, and M. Miyamoto, *Chem. Pharm. Bull., Tokyo*, 1972, **20**, 2291.

a parallel reaction with 3-methoxy-4-methoxymethoxycinnamoylmethane (29) gave only traces of carboxylic acid product (30).



(27) $R^1 = R^2 = R^3 = H$
(28) $R^1 = R^2 = H, R^3 = CO_2H$
(29) $R^1 = MeOCH_2O, R^2 = MeO, R^3 = H$
(30) $R^1 = MeOCH_2O, R^2 = MeO, R^3 = CO_2H$

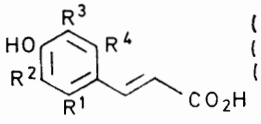
It is pertinent to refer here to some biosynthetic experiments [10,11] on the 9-phenylphenalen-1-ones haemocorin (14) and lachnanthoside (15). Tyrosine, phenylalanine, and [1-] and [2-^{14}C]sodium acetate are incorporated into (14) in *Haemodorum corymbosum* [10] (0·5, 0·3, 0·09, and 0·23% respectively), and into (15) in *Lachnanthes tinctoria* (0·5, 3·5, 0·2, and 0·6% respectively). These



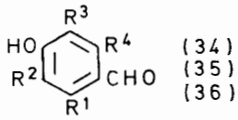
SCHEME 4

results are interpreted in favour of biosynthesis as summarised in path *a* (Scheme 4), with tyrosine and phenylalanine supplying different rings (A and B respectively). [1-^{14}C]Tyrosine labelled C-5 specifically. No degradations were effected on the products after feeding phenylalanine which may therefore also have labelled ring A; phenylalanine is certainly incorporated into *p*-hydroxyaromatic rings, *e.g.* in bis-(4-hydroxycinnamoyl)methane (3) (see below). Further, the sites of incorporation from acetate were not located, and no significance was attached to the incorporations of [1-^{14}C]acetate. Although the correct conclusions may indeed have been drawn from these experiments, the results appear to be equally consistent with Scheme 4, path *b*, in which ring B has a polyketide origin.

A different set of experiments were initiated to investigate the role of cinnamic acids in curcumin biosynthesis, and to find acceptable oxygenation patterns for such precursors. Accordingly, we prepared [5-^{3}H]ferulic acid (31), [2,5-^{3}H]caffeic acid (32), and [3-^{3}H]coumaric acid (33). As a preliminary experiment, the base-catalysed deuteriations of vanillin, 3,4-dihydroxybenzaldehyde, and 4-hydroxybenzaldehyde were examined, the yields of [5-^{2}H]vanillin (34), [2,5-$^2H_2$]-3,4-dihydroxybenzaldehyde (35), and [3-^{2}H]-4-hydroxybenzaldehyde (36) were optimised, and the sites of isotope exchange checked by n.m.r. The corresponding tritiated benzaldehydes were made, and converted into the corresponding cinnamic acids (31)—(33) by condensation [23] with malonic acid; no label was lost during the reaction. Direct deuteriation of ethyl ferulate (triethylamine catalysed) gave rise to exchange both in the ring (*ortho* to hydroxy) and in the double bond (α to carbonyl). The same positions, and the methyl protons, were exchanged in feruloylmethane (23), in a similar reaction. The aldehyde (34) was used to make feruloylmethane labelled only in the ring, while



(31) $R^1 = R^4 = H, R^3 = {}^3H, R^2 = OMe$
(32) $R^4 = H, R^1 = R^3 = {}^3H, R^2 = OH$
(33) $R^1 = R^3 = R^4 = H, R^2 = {}^3H$



(34) $R^1 = R^4 = H, R^3 = {}^2H$ or $^3H, R^2 = OMe$
(35) $R^4 = H, R^1 = R^3 = {}^2H$ or $^3H, R^2 = OH$
(36) $R^1 = R^3 = R^4 = H, R^2 = {}^2H$ or ^3H

tritiated bis-(4-hydroxycinnamoyl)methane (3) was prepared from (36). Purification of all these tritiated phenols was carried out by preparative paper chromatography.

The results of the first experiments (13—15) in administration of [^{3}H]cinnamic acids to *C. longa* are given in Table 3A—C. Both ferulic and coumaric acids appear to be more acceptable precursors to curcumin than caffeic acid. However, since the incorporation differences were not great, the results might have reflected individual plant differences. The experiments were therefore repeated (16—18), but with the refinement that [1-^{14}C]phenylalanine was co-administered with each [^{3}H]cinnamic acid (Table 3A). Doubly-labelled curcumin and its relatives (2) and (3) were isolated from the rhizomes (Table 3B) and incorporations for both [^{3}H] and [^{14}C] precursors were calculated (Table 3C). ^{3}H/^{14}C Incorporation ratios (Table 3D) then provide an improved means of comparing results from experiments using different plants. Reference to Table 3D shows that none of the cinnamic acids was quite as well incorporated as phenylalanine into (1), (2), or (3) (although the differences are not great in some cases) suggesting that free cinnamic acids may not be involved in biosynthesis. Caffeic acid was a relatively unacceptable precursor to all pigments (1)—(3), and its participation may be discounted. In the cases of curcumin and 4-hydroxycinnamoyl(feruloyl)-methane (2), the figures at their face value seem to show ferulic acid to be a slightly better precursor than 4-coumaric acid; however this difference is not significant when account is taken of the fact that [3-^{3}H]coumaric acid may lose tritium on incorporation into (1) and (2), and a multiplication factor (not greater than two) may need to be applied to the appropriate incorporations in

[23] R. Adams and T. E. Birckstahler, *J. Amer. Chem. Soc.*, 1952, **74**, 5346.

TABLE 3A

Administration of [^{3}H]cinnamic acids and [1-^{14}C]phenylalanine to *C. longa*

Experiment no.	Precursor	Activity (μCi)
13	[^{3}H]Ferulic acid	884
14	[^{3}H]Caffeic acid	835
15	[^{3}H]Coumaric	775
16 *	[^{3}H]Ferulic acid	80
	[1-^{14}C]Phenylalamine	17
17 *	[^{3}H]Caffeic acid	81
	[1-^{14}C]Phenylalanine	17
18 *	[^{3}H]Coumaric acid	70
	[1-^{14}C]Phenylalanine	17

* Both precursors administered to the same plant.

TABLE 3B

Products from administration of [^{3}H]cinnamic acids and [1-^{14}C]phenylalanine

	Compound					
	(1)		(2)		(3)	
Experiment no.	Isolated (mg)	Added (mg)	Isolated (mg)	Added (mg)	Isolated (mg)	Added (mg)
13	13·0	23·7				
14	11·1	26·1				
15	26·1	11·0				
16	22·1	14·0	4·0	31·8	5·2	30·9
17	34·4	0	3·7	32·6	5·0	31·8
18	11·8	24·8	9·4	25·0	13·7	26·5

TABLE 3C

Activities of products from administration of [^{3}H]cinnamic acids and [1-^{14}C]phenylalanine

	Expt. 16		Expt. 17		Expt. 18	
Metabolite	Specific activity (disint. min^{-1} mM^{-1})	Incorporation (%)	Specific activity (disint. min^{-1} mM^{-1})	Incorporation (%)	Specific activity (disint. min^{-1} mM^{-1})	Incorporation (%)
[^{3}H]-(1)	2·53 × 10^5	0·014	3·78 × 10^5	0·020	2·41 × 10^5	0·015 *
[^{14}C]-(1)	9·01 × 10^4	0·023	4·74 × 10^5	0·117	1·57 × 10^5	0·041
[^{3}H]-(2)	1·70 × 10^5	0·010	1·38 × 10^5	0·008	2·58 × 10^5	0·017 *
[^{14}C]-(2)	4·76 × 10^4	0·013	1·70 × 10^5	0·048	1·17 × 10^5	0·032
[^{3}H]-(3)	1·19 × 10^5	0·008	8·51 × 10^4	0·006	2·20 × 10^5	0·018
[^{14}C]-(3)	4·85 × 10^4	0·015	3·07 × 10^5	0·097	1·31 × 10^5	0·045

Metabolite	Specific activity (disint. min^{-1} mM^{-1})	Incorporation (%)	
[^{3}H]-(1)	1·55 × 10^7	0·079	Expt. 13
[^{3}H]-(1)	9·67 × 10^5	0·005	Expt. 14
[^{3}H]-(1)	3·80 × 10^6	0·022 *	Expt. 15

* These figures are not corrected for loss of tritium during aryl hydroxylation in biosynthesis; the *maximum* correction factor would be × 2.

TABLE 3D

Ratios of incorporations of [^{3}H]cinnamic acids and [1-^{14}]phenylalanine

Metabolite	Expt. 16 [^{3}H]/[^{14}C] Incorporation ratio	Expt. 17 [^{3}H]/[^{14}C] Incorporation ratio	Expt. 18 [^{3}H]/[^{14}C] Incorporation ratio
(1)	0·61	0·17	0·37 *
(2)	0·77	0·17	0·53 *
(3)	0·53	0·06	0·40

* Not corrected for loss of tritium during incorporation.

Tables 3C and D. For bis-(4-hydroxycinnamoyl)methane, the curious result emerged that ferulic acid appeared to be a better precursor than 4-coumaric acid; further experimentation is needed before this can be understood.

Finally, we record that neither [^{3}H]feruloylmethane nor [^{3}H]bis-(4-hydroxycinnamoyl)methane were significantly incorporated into curcumin.

EXPERIMENTAL

Unless otherwise stated, the following generalisations apply. U.v. spectra were measured in ethanol; $\log_{10}$ ε follows $\lambda_{max.}$; n.m.r. spectra were recorded in deuteriochloroform, with tetramethylsilane as internal standard; assignments of hydroxy-protons were checked by deuterium exchange. M.p.s were measured with a hot-stage microscope. Column chromatography used silica gel M.F.C. (Hopkin and Williams); thin layer chromatography employed silica gel G or HF_{254} (Merck–Stahl). Specific activities were measured by liquid scintillation, with a Nuclear Enterprises NE 8130 spectrometer and using dioxan- or toluene-based scintillators (NE 250 and NE 233). Counting efficiencies were determined using [^{14}C] or [^{3}H]toluene as internal standard.

Extraction of Turmeric.—Dried, crushed rhizomes (1 kg) of *Curcuma longa* were extracted in a Soxhlet apparatus with light petroleum (b.p. 40—60°) (3 dm³) for 24 h. The extraction was continued with benzene (3 dm³) for 72 h. Evaporation of the benzene extracts gave an orange powder (10·6 g). T.l.c. examination (chloroform–ethanol, 25 : 1) indicated the presence of 3 components. Separation was effected by p.l.c. using 18 in × 18 in plates, each carrying silica (100 g). The extract (3 g) was applied to 6 plates, and eluted with chloroform–ethanol (25 : 1). Three orange bands separated, and were removed and extracted with acetone. The top band gave, after evaporation, curcumin (1) (1·46 g), m.p. 184—185° (from ethanol) (lit.,[14] 184—186°), (Found: C, 68·5; H, 5·4. Calc. for $C_{21}H_{20}O_6$: C, 68·5; H, 5·45%), M^+ 368, $\lambda_{max.}$ 268 (4·09) and 430 nm (4·74), τ [$(CD_3)_2CO$] 2·42 (2H, d, *J* 16 Hz, 4,4′-H_2), 2·70 (2H, d, *J* 2 Hz, 6,6′-H_2), 2·84 (2H, dd, *J* 2 and 8 Hz, 10,10′-H_2), 3·14 (2H, d, *J* 8 Hz, 9,9′-H_2), 3·34 (2H, d, *J* 16 Hz, 3,3′-H_2), 4·04 (1H, s, 1-H), and 6·09 (6H, s, 2 × OMe), τ [$(CD_3)_2CO$ at −90 °C] 0·61 (2H, ArOH) and −7·04 (1H, enol OH). The middle band provided 4-hydroxycinnamoyl(feruloyl)methane (2) (0·53 g), m.p. 168—169° (from benzene) (lit.,[14] 172·5—174·5°) (Found: C, 71·2; H, 5·65. Calc. for $C_{20}H_{18}O_5$: C, 71·0; H, 5·35%), M^+ 338, $\lambda_{max.}$ 251 (4·13) and 423 nm (4·71), τ [$(CD_3)_2CO$], 2·40 (2H, d, *J* 16 Hz, 4,4′-H_2), 2·46 (2H, d, *J* 8 Hz, 6′,10′-H_2), 2·70 (1H, d, *J* 2 Hz, 6-H), 2·84 (1H, dd, *J* 2 and 8 Hz, 10-H), 3·12 (2H, d, *J* 8 Hz, 7′,9′-H_2), 3·14 (1H, d, *J* 8 Hz, 9-H), 3·34 and 3·38 (both 1H, d, *J* 16 Hz, 3 and 3′-H), 4·04 (1H, s, 1-H), and 6·09 (3H, s, OMe), τ [$(CD_3)_2CO$ at −90 °C] −6·82 (enol OH), 0·34 (ArOH), and 1·06 (ArOH). Finally, the lowest band yielded bis-(4-hydroxycinnamoyl)methane (0·7 g) as an orange glassy solid, (Found: M^+ 308·1055. Calc. for $C_{19}H_{16}O_4$: 308·1049), $\lambda_{max.}$ 248 (4·07) and 418 nm (4·57), τ [$(CD_3)_2CO$] 2·40 (2H, d, *J* 16 Hz, 4,4′-H_2), 2·46 (4H, d, *J* 8 Hz, 6,6′,10,10′-H_4), 3·10 (4H, d, *J* 8 Hz, 7,7′,9,9′-H_4), 3·38 (2H, d, *J* 16 Hz, 3,3′-H_2), and 4·04 (1H, s, 1-H), τ [$(CD_3)_2CO$ at −90 °C] −6·82 (enol OH) and 0·32 (ArOH).

Diacetylcurcumin.—Curcumin (0·67 g) in 10% aqueous sodium hydroxide (5 cm³) was shaken with acetic anhydride (0·34 cm³) and crushed ice (10 g) for 5 min. The yellow precipitate was collected, washed, dried, and crystallised from ethanol to yield diacetylcurcumin (19) (0·55 g, 67%). m.p. 170° (lit.[14] 170—172°) (Found: C, 66·7; H, 5·55. Calc. for $C_{25}H_{24}O_8$: C, 66·35; H, 5·3%), M^+ 452, $\lambda_{max.}$ 255 (4·10) and 402 nm (4·63), $\nu_{max.}$ ($CHCl_3$) 1760 cm⁻¹, τ 2·34 (2H, d, *J* 16 Hz, 4,4′-H_2), 2·6—3·1 (6H, m, ArH), 3·44 (2H, d, *J* 16 Hz, 3,3′-H_2), 4·12 (1H, s, 1-H), 6·14 (6H, s, 2 × OMe), and 7·70 (6H, s, 2 × COMe).

Methylation of Curcumin.—(*a*) Curcumin (0·86 g) in dry acetone (50 cm³) with methyl iodide (10 cm³) was refluxed over anhydrous potassium carbonate (1 g) for 24 h with stirring. After filtration the solution was evaporated, and the residue was extracted with ether. Evaporation of the ether extracts gave *tetramethylcurcumin* [1,7-*bis*-(3,4-*dimethoxyphenyl*)-4,4-*dimethylhepta*-1,6-*diene*-3,5-*dione*] (17), (0·74 g, 75%), m.p. 137·5—138·5° (from methanol) (Found: C, 70·75; 6·5. $C_{25}H_{28}O_6$ requires C, 70·75; H, 6·6%), M^+ 424, $\lambda_{max.}$ 252 (4·09) and 350 nm (4·34), $\nu_{max.}$ (KBr) 1690 and 1667 cm⁻¹, τ 2·36 (2H, d, *J* 16 Hz, 4,4′-H_2), 2·90 (2H, dd, *J* 2 and 8 Hz, 10,10′-H_2), 3·02 (2H, d, *J* 2 Hz, 6,6′-H_2), 3·20 (2H, d, *J* 8 Hz, 9,9′-H_2), 3·39 (2H, d, *J* 16 Hz, 3,3′-H_2), 6·14 (12H, s, 4 × OMe), and 8·53 (6H, s, CMe_2).

(*b*) In a similar preparation, the *trimethyl derivative* [1,7-*bis*-(3,4-*dimethoxyphenyl*)-4-*methylhepta*-1,6-*diene*-3,5-*dione*] (18) was obtained, m.p. 150—152° (from methanol) (Found: C, 70·5; H, 6·7. $C_{24}H_{26}O_6$ requires C, 70·25; H, 6·35%), M^+ 410, $\lambda_{max.}$ 264 (4·10) and 437 nm (4·59), τ −7·32 (1H, s, OH), 2·33 (2H, d, *J* 16 Hz, 4,4′-H_2), 2·84 (2H, dd, *J* 2 and 8 Hz, 10,10′-H_2), 2·94 (2H, d, *J* 2 Hz, 6,6′-H_2), 3·06 (2H, d, *J* 16 Hz, 3,3′-H_2), 3·15 (2H, d, *J* 8 Hz, 9,9′-H_2), 6·08 and 6·10 (both 6H, s) (4 × OMe), and 7·83 (3H, s, 1-Me).

(*c*) Curcumin (0·43 g) in dry benzene (100 cm³) was refluxed with dimethyl sulphate (10 cm³) over anhydrous potassium carbonate (1 g) for 48 h, with stirring. After evaporation of benzene, the residue was stirred with warm aqueous sodium hydrogen carbonate. The mixture was extracted with chloroform, and the extracts were washed, dried, and evaporated. The residue was purified by p.l.c. (chloroform–ethanol, 50 : 1). The major band (highest R_F) gave, after extraction with chloroform, *dimethylcurcumin* [1,7-*bis*-(3,4-*dimethoxyphenyl*)*hepta*-1,6-*diene*-3,5-*dione*] (16), m.p. 128—130° (from ethanol) (Found: C, 69·85; H, 6·45. $C_{23}H_{24}O_6$ requires C, 69·7; H, 6·05%), M^+ 396, $\lambda_{max.}$ 262 (4·08) and 420 nm (4·66), τ 2·42 (2H, d, *J* 16 Hz, 4,4′-H_2), 2·88 (2H, dd, *J* 2 and 8 Hz, 10,10′-H_2), 2·94 (2H, d, *J* 2 Hz, 6,6′-H_2), 3·16 (2H, d, *J* 8 Hz, 9,9′-H_2), 3·54 (2H, d, *J* 16 Hz, 3,3′-H_2), 4·20 (1H, s, 1-H), and 6·10 (12H, s, 4 × OMe).

Hydrogenation of Curcumin.—Curcumin (1 g) in ethyl acetate (100 cm³) was hydrogenated overnight at 100 °C and 50 atm over 10% palladium–charcoal catalyst. The filtered solution was concentrated and separated by p.l.c. (chloroform–ethanol, 25 : 1). Two major bands were detected. The upper band, after the usual extraction, gave tetrahydrocurcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-heptane-3,5-dione] (20), (0·4 g), m.p. 95—96° [from light petroleum (b.p. 60—80°)], (lit.,[24] 96°) (Found: C, 67·65; H, 6·4. Calc. for $C_{21}H_{24}O_6$: C, 67·75; H, 6·45%), M^+ 372, τ 3·19 (2H, d, *J* 8 Hz, 10,10′-H_2), 3·34 (2H, s, 6,6′-H_2), 3·39 (2H, d, *J* 8 Hz, 9,9′-H_2), 4·46 (2H, ArOH), 4·60 (1H, s, 1-H), 6·17 (6H, s, 2 × OMe), and 7·0—7·6 (8H, m). The lower band afforded *hexahydrocurcumin* [1,7-*bis*-(4-*hydroxy*-3-*methoxyphenyl*)-5-*hydroxyheptane*-3-*one*] (21) (0·4 g), m.p. 78—80° [from light petroleum (b.p. 40—60°)] (Found: C, 67·3; H, 6·8. $C_{21}H_{26}O_6$ requires C, 67·4; H, 6·95%), M^+ 374, τ 3·21 (2H, d, *J* 8 Hz, 10,10′-H_2), 3·35 (2H, s, 6,6′-H_2), 3·39 (2H, d, *J* 8 Hz, 9,9′-H_2), 4·28 (2H, ArOH), 5·98 (1H, m, 2-H), 6·19 (6H, s, 2 × OMe), 7·0—7·6 (8H, m, 1,3′,4,4′-H_8), and 8·32 (2H, m, 3-H).

Synthesis of Curcumin and Derivatives.—The following procedure was typical. The aromatic aldehyde (0·1 mol) was dissolved in dry ethyl acetate (50 cm³) and tri-(s-butyl) borate (46 g, 0·2 mol; prepared by heating boric acid with 2-butanol with azeotropic removal of water, b.p. 78—80° at 13 mmHg) was added. The complex formed by shaking acetylacetone (5 g, 0·05 mol) with boric oxide (2·5 g, 0·035 mol) for 1 h was also added, and the mixture stirred for 5 min. n-Butylamine (1 cm³) was added dropwise during 40 min; after further stirring for 4 h, the solution was set aside overnight. 0·4N-Hydrochloric acid (75 cm³) at 60° was then added, and the mixture stirred for 1 h before extraction with ethyl acetate. The washed extracts were evaporated and the residual paste stirred with dil. hydrochloric acid for 1 h. The solid product was collected, washed, dried, and recrystallised. In this way the following compounds were prepared. (a) Curcumin, m.p. 184—186°, 78%, from vanillin (15·2 g, 0·1 mol); (b) 4-hydroxycinnamoyl(feruloyl)methane, m.p. 169—171°, 43% (isolated

[24] W. Lampe and J. Smolinska, *Bull. Acad. polon. Sci.*, 1958, **6**, 481.

using p.l.c.), from vanillin (7·6 g, 0·05 mol) and 4-hydroxybenzaldehyde (6·1 g, 0·05 mol); (c) bis-(4-hydroxycinnamoyl)-methane, m.p. 224—226°, 68%, from 4-hydroxybenzaldehyde (7·3 g, 0·06 mol); (d) diacetyl curcumin, m.p. 170—171°, 54%, from acetylvanillin (11·64 g, 0·06 mol); (e) dimethylcurcumin, m.p. 129—130°, 73%, from veratraldehyde (16·6 g, 0·1 mol); (f) [^{3}H]-bis-(4-hydroxycinnamoyl)-methane (34%) (74 mCi mM^{-1}), and [^{3}H]-hydroxycinnamoylacetone (23%) (44 mCi mM^{-1}), from [^{3}H]-4-hydroxybenzaldehyde (70 mg, 49 mCi mM^{-1}).

Feruloylacetone.—The complex from acetylacetone (7·5 g) and boric oxide (3·75 g) was suspended in dry ethyl acetate (50 cm^3) with tri-(s-butyl) borate (23 g) at 0 °C. Vanillin (3·75 g) and n-butylamine (0·5 cm^3) were added to the stirred suspension during 90 min. After stirring for 90 min more, the reaction mixture was set aside overnight. 0·4N-Hydrochloric acid (50 cm^3) at 60° was added. The mixture was stirred for 1 h and then extracted with ethyl acetate. The extracts were washed, dried, and concentrated, before separation by p.l.c. (chloroform–ethanol, 25 : 1). Two main bands were observed; that of lower R_F (orange) afforded curcumin, while the band of higher R_F (yellow) gave, after extraction, feruloylacetone (1·97 g, 34%), m.p. 146—147°, (lit.,[25] 143—145°).

Degradation of Curcumin by Aqueous Alkali.—Curcumin (30 mg) was refluxed for 90 min in 5% aqueous sodium hydroxide (25 cm^3). The mixture was then distilled during 45 min, when *ca.* 12 cm^3 distillate had been collected. Sodium hydroxide (500 mg) was added to the distillate, followed by potassium iodide (0·4 g) and iodine (0·2 g) in water (2 cm^3). Iodoform (12·2 mg, 38%) was collected by centrifugation, washed, and dried for 4 h at *ca.* 15 mmHg. Crystallisation from aqueous acetone gave yellow needles, m.p. 121—123 °C. The residue from distillation was acidified with conc. hydrochloric acid (5 cm^3) and extracted with ethyl acetate (2 × 10 cm^3) and chloroform (2 × 10 cm^3). The combined extracts were dried and concentrated before separation by p.l.c. (silica gel HF_{254}) (benzene–dioxan–acetic acid, 36 : 10 : 1). The two bands resolved were washed from the silica with chloroform. The upper band gave vanillin (9·4 mg, 76%), and the other band provided ferulic acid (11·3 mg, 71%). The latter was purified by rechromatography in the same solvent system, followed by sublimation at 125° and 0·01 mmHg. The former was rechromatographed using chloroform–ethanol (25 : 1) and sublimed at 50° and 0·01 mmHg. The solid products had m.p.s and mixed m.p.s, and spectra identical with authentic samples. Ferulic acid formed a *p-bromobenzylpseudothiuronium salt* by the standard method, m.p. 144—145° (Found: C, 49·15; H, 4·3; N, 5·9. $C_{18}H_{19}BrN_2O_4S$ requires C, 49·2; H, 4·35; N, 6·35%).

Oxidation of Vanillin.—Silver oxide [freshly prepared from silver nitrate (0·24 g) in water (2 cm^3) and sodium hydroxide (0·1 g) in water (1 cm^3)] was suspended in water (4 cm^3) containing sodium hydroxide (0·4 g) and the mixture was heated to 60°. Vanillin (100 mg) was added, and the mixture was stirred at 60° for 20 min. The solution was filtered and the residue washed with hot water (4 cm^3). The solution was decolourised with sulphur dioxide, and acidified with conc. hydrochloric acid (1 cm^3). The precipitate was collected in ether. The ethereal solution, after drying and evaporation, gave vanillic acid (98 mg, 88%), identical with an authentic sample.

Decarboxylation of Vanillic Acid.—Vanillic acid (10 mg) was heated in quinoline (5 cm^3) with copper powder (100 mg) at 220—230 °C for 2 h. Nitrogen (carbon dioxide free) was passed through the mixture continuously and swept through saturated aqueous barium hydroxide traps. After decantation of excess of solution, precipitated barium carbonate was collected from the traps by centrifugation (60 mg, 51%, after washing and drying). In experiments with radioactive material, barium carbonate (20—80 mg) was finely powdered, and counted in suspension in a gel, prepared from toluene-based scintillator (10 cm^3) and Cab-O-sil gelling agent (0·55 g), using ultrasonic dispersion.

Decarboxylation of Ferulic Acid.—Ferulic acid (100 mg) was treated as above for 30 min. Barium carbonate (92 mg, 90%) was obtained.

Oxidation of Curcumin.—Curcumin (300 mg) in dioxan (15 cm^3) and water (5 cm^3) was treated with osmium tetroxide (12 mg), and the solution stirred (15 min). Sodium periodate (2·2 g) was added over 15 min; stirring of the reaction mixture was continued for 24 h. The filtered solution was extracted with chloroform (4 × 15 cm^3). Vanillin (92 mg, 37%) was isolated from the concentrated extracts using p.l.c. (chloroform–ethanol, 25 : 1), identical with an authentic specimen.

Oxidation of Tetramethylcurcumin.—Tetramethylcurcumin (275 mg) was treated as in the previous experiment, but using osmium tetroxide (7 mg), and sodium periodate (1·2 g). Isolation of products in a similar fashion yielded veratraldehyde (147 mg, 67%), identified by comparison with authentic material.

[5-^{2}H]- *and* [5-^{3}H]-*Vanillin*.—In general, the method of Kirby and Ogunkoya [26] was employed. Vanillin (100 mg), deuterium oxide (0·5 cm^3), and triethylamine (0·09 cm^3) were sealed under nitrogen and heated at 100 °C for 3 days. [5-^{2}H]Vanillin was isolated from the acidified product through chloroform extraction and purified by chromatography on silica (91 mg, 91%), τ (ArH signals) 1·92 (1H, d, *J* 2 Hz) and 2·08 (1H, d, *J* 2 Hz). A similar experiment, but using tritiated water (0·1 cm^3; 4·4 Ci cm^{-3}) afforded [5-^{3}H]-vanillin (89%). Labile tritium was removed by repeated treatment with methanol. Purification to constant activity (38 mCi mM^{-1}) was effected by p.l.c. (chloroform–ethanol, 25 : 1) and resublimation.

[5-^{2}H]- *and* [5-^{3}H]-*Ferulic Acid*.—[5-^{2}H]Vanillin (50 mg) and malonic acid (69 mg) in pyridine (100 mm^3) and aniline (10 mm^3) were heated at 55° for 14 h. The product was shaken with ice (1 g) and conc. hydrochloric acid (0·5 cm^3). [5-^{2}H]Ferulic acid (35 mg, 55%) was filtered off and crystallised from water, m.p. 170—172°, τ [$(CD_3)_2SO$] 2·42 (1H, d, *J* 16 Hz), 2·68 (1H, d, *J* 2 Hz), 2·87 (1H, d, *J* 2 Hz), 3·61 (1H, d, *J* 16 Hz). A similar preparation using [5-^{3}H]vanillin (90 mg) gave [5-^{3}H]ferulic acid (90 mg, 78%), recrystallised from water to constant activity, 35 mCi mM^{-1}.

[2,5-$^2H_2$]- *and* [2,5-$^3H_2$]-3,4-*Dihydroxybenzaldehyde*.—3,4-Dihydroxybenzaldehyde (276 mg), triethylamine (0·28 cm^3) and deuterium oxide (1·5 cm^3) were heated at 100° for 3 days in a sealed tube under nitrogen. The deuteriated product was isolated from the acidified mixture by ether extraction and resublimed (227 mg, 81%), τ (MeOH) 2·66 (1H). A similar preparation with the aldehyde (100 mg), triethylamine (0·1 cm^3), and tritiated water (0·1 cm^3; 4·4 Ci cm^{-3}) gave the tritiated aldehyde. Labile tritium was removed by methanol treatment. Specific activity after resublimation was 57 mCi mM^{-1}.

[2,5-$^2H_2$]- *and* [2,5-$^3H_2$]-*Caffeic Acid*.—The method

[25] V. Lampe, *Ber.*, 1918, **51**, 1347.
[26] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.

described for ferulic acid was used, but with [2,5-$^2H_2$]-3,4-dihydroxybenzaldehyde (100 mg) and malonic acid (151 mg) as the solid reactants. [2,5-$^2H_2$]-Caffeic acid (104 mg, 80%), m.p. 222—223° (from water) was obtained, τ [$(CD_3)_2SO$] 2·51 (1H, d, *J* 16 Hz), 3·00 (1H, s), and 3·79 (1H, d, *J* 16 Hz). Similarly [2,5-$^3H_2$]caffeic acid (85%) was obtained, 58 mCi mM^{-1}.

[^{2}H]- *and* [^{3}H]-*Exchange of 4-Hydroxybenzaldehyde*.—4-Hydroxybenzaldehyde (244 mg) was treated in a similar way to 3,4-dihydroxybenzaldehyde (above). The product (204 mg, 84%) was isolated and purified, as above, τ (MeOH) 2·18 (s), 2·18 (d, *J* 8 Hz), and 3·05 (d, *J* 8 Hz); integration indicated *ca.* 50% 3-deuteriation. A similar reaction with the aldehyde (100 mg), triethylamine (0·114 cm^3), and tritiated water (0·1 cm^3, 4·4 mCi cm^{-3}) gave [3-^{3}H]-4-hydroxybenzaldehyde (82 mg, 47 mCi mM^{-1}).

[3-^{3}H]*Coumaric Acid*.—[3-^{3}H]-4-Hydroxybenzaldehyde (80 mg) and malonic acid (137 mg) were heated together in pyridine (100 mm^3) and aniline (10 mm^3) at 65° for 7·5 h. After acidification, 4-coumaric acid was filtered off (95 mg, 88%), 50 mCi mM^{-1} after repeated crystallisation from water.

Deuteriation of Ethyl Ferulate and Feruloylmethane.—Ethyl ferulate (0·5 g), deuterium oxide (2 cm^3), triethylamine (0·31 cm^3), and dioxan (2 cm^3) were heated in a sealed tube at 100° for 3 days. Feruloylmethane (100 mg) with deuterium oxide (0·5 cm^3) and triethylamine was similarly treated. In both cases the phenols were recovered by solvent extraction (ether, chloroform) from the acidified, cooled product. [^{2}H]Ethyl ferulate had τ [$(CD_3)_2SO$] 2·42 (1H, s, CH=CD·CO), 2·66 (1H, d, *J* 2 Hz, 2H), 2·87 (1H, d, *J* 2 Hz, 6H), 5·81 (2H, q), 6·16 (3H, s), and 8·74 (3H, t). [^{2}H]Feruloylmethane had τ [$(CD_3)_2SO$] 2·43 (1H, s, CH=CD·CO), 2·69 (1H, d, *J* 2 Hz, 2H), 2·85 (1H, d, *J* 2 Hz, 6H), and 6·17 (3H, s).

1-(3-*Methoxy*-4-*methoxymethoxyphenyl*)*but*-1-*en*-3-*one* (29).—Feruloylmethane (9·6 g) was dissolved in methanol (130 cm^3) containing sodium (1·15 g). The solution was evaporated to dryness and the residue stirred under reflux with benzene (120 cm^3) and chloromethyl methyl ether (3·8 cm^3), for 3 h. After cooling and filtration the benzene solution was washed with 2% aqueous sodium hydroxide, dried, and evaporated to yield the *dimethoxy-compound* (29) (6·5 g, 55%), m.p. 71—73° (Found: C, 66·3; H, 6·7. $C_{13}H_{16}O_4$ requires C, 66·1; H, 6·8%).

Labelled Precursor Incorporation Experiments.—Dormant rhizomes of *C. longa* (from Bombay) were kept at 35° in a moist atmosphere until growth began (4—8 weeks). Plants reached *ca.* 18 in in 3—4 months, and were fed when new rhizomes began to form. Precursors were supplied in aqueous solution (0·5—1 cm^3) (phenols were neutralised with the calculated quantity of sodium hydroxide), using a cotton wick inserted in the stem 1—2 in above the root stock. After the required period the whole root stock was washed, and the new rhizomes * were removed and immersed in liquid nitrogen for 5 min before crushing to powder at −60°. The powder was dried overnight at 65°, and then stored at −25° until required for extraction. Curcumin was extracted, diluted with synthetic material, and degraded, as described above. Radioactive curcumin was decolourised before counting; the sample (*ca.* 1 mg) was treated with sodium borohydride (10 mg) in dioxan (0·8 cm^3) and water (0·2 cm^3) for 2 h. Count rates for such samples rose a little over a few hours and then remained at a constant maximum for *ca.* 12 h, when the counting efficiency began to fall.

[3/1119 *Received* 31*st*, *May*, 1973,]

* Incorporations of labelled precursors into curcumin in old rhizomes were negligible, suggesting little or no turnover of this compound at these sites.