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Diarylheptanoids; The Problems of the Biosynthesis

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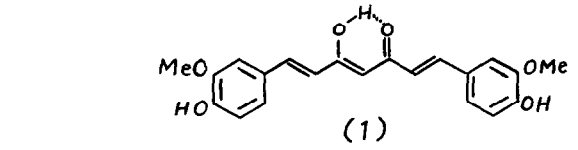
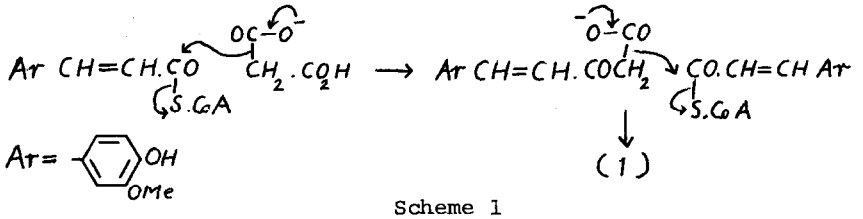
Attention has recently been drawn[1] to various natural products containing a 1,7-diarylheptane skeleton, which apparently form a group[2-6] {the 'diaryl heptanoids'} biogenetically related to curcumin[6] (1)*, the pigment of Curcuma longa rhizome (turmeric). Haemocorin[7] may also be related to this group. The biosynthesis[8] of curcumin, would plausibly appear to be related to that of lignans, involving the union of two cinnamate units with a central methylene supplied by malonate, (Scheme 1). In order to examine this proposition we have undertaken tracer experiments, which have had an unexpected outcome.



(1)

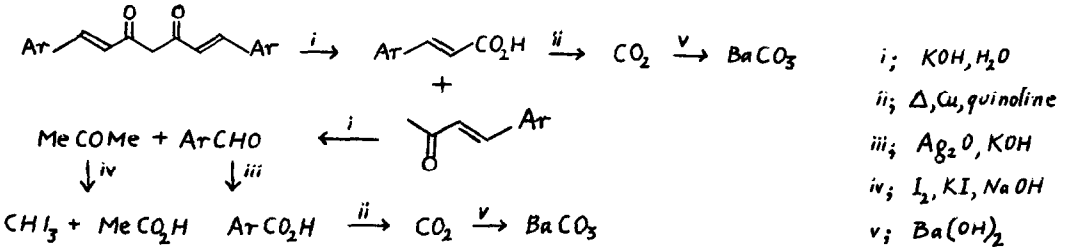


Scheme 1

Natural curcumin, m.p. 184-185°, was obtained from dried C. longa rhizome, and the degradation shown in Scheme 2 worked out, as a means of assessing relative radioactivity in each acyclic carbon, and the aromatic rings.

*Curcumin (1) is shown as the enol as indicated by spectroscopy; in the schemes, the ketonic form is shown to emphasize symmetry.

C. longa plants (India) were employed in experiments in which sodium 2-^{14}C acetate, sodium 2-^{14}C malonate, 1- and 3- ^{14}C phenylalamine, and sodium ^{3}H-ferulate (prepared by malonic acid condensation from vanillin tritiated with T_2O- Et_3N[9]) were administered by the wick method to individual plants, during 6 days. Experiments were timed, as far as possible, to coincide with the few weeks of intense metabolic activity in which new rhizomes are formed, each containing 5-25mg curcumin. Close quantitative comparisons between similar experiments in different plants are not expected under these conditions. The results are shown in the Table.



Scheme 2

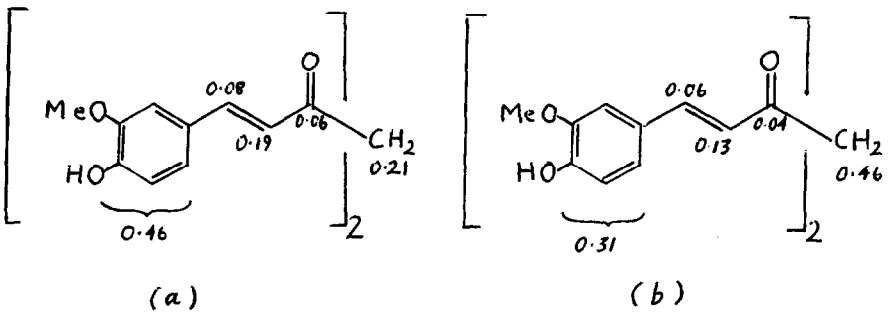


Fig. Fractional distribution of activity in curcumin from (a) sodium 2-^{14}C acetate and (b) sodium 2-^{14}C malonate feeding.

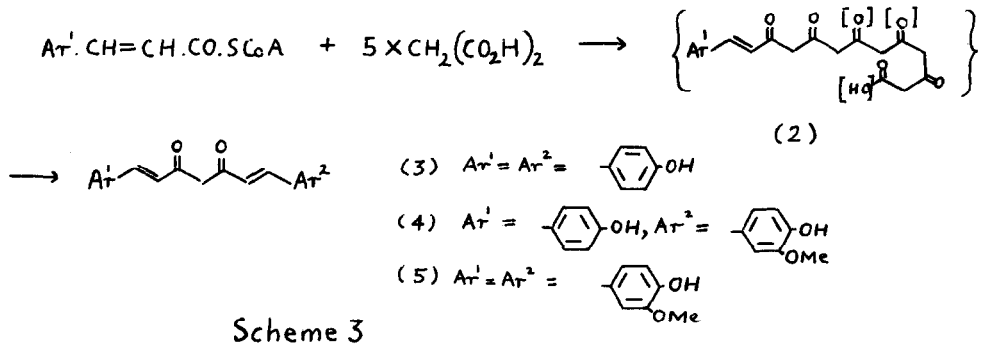
Table. Labelled precursor incorporations in Curcuma longa

1 Precursor	2 Activity of curcumin (dpm/m mol)	3 % Incorporation	Activity of degradation products (dpm/m mol) 4 Vanillin	5 $BaCO_3$ from Vanillic Acid	6 Ferulic Acid	7 $BaCO_3$ from Ferulic Acid	8 Iodoform
Na2-^{14}C Acetate (0.1mc)	3.22×10^5	1.3×10^{-2}	1.03×10^5	1.508×10^4	1.517×10^5	1.178×10^4	5.78×10^4
Na2-^{14}C Malonate (0.1mc)	6.54×10^5	2.1×10^{-2}	1.29×10^5	1.988×10^4	1.874×10^5	1.324×10^4	1.821×10^5
1-^{14}C Phenylalanine (0.1mc)	*2.721×10^6	1.03×10^{-1}	1.288×10^4		2.28×10^6		0.0
3-^{14}C Phenylalanine (0.1mc)	1.361×10^6	5.1×10^{-2}					
Na^{3}H Ferulate (3.6μc)	5.34×10^3	1.7×10^{-2}					

* Close balance of activity was not attained, on degradation, in this experiment, but location of the label is unambiguous.

These incorporation figures (column 3) indicate that all these precursors are implicated in the biosynthesis, and it is clear that a phenylalanine derived precursor must supply at least one C_9 unit. On degradation of curcumin obtained after feeding 1-^{14}C phenylalanine >99% of activity was found to be located in the carboxyl of ferulic acid, showing that no unexpected rearrangement of the phenylalanine side chain occurred.

However, on feeding labelled acetate and malonate, we were surprised to find that the majority of the radioactivity was not confined to the central methylene. The detailed fractional distribution of activity in these two experiments is shown in the Figure; it is of course impossible to distinguish between the two symmetry-related C_9 parts of curcumin, and thus the total activity for each pair of atoms or groups is shown. Acetate and malonate supply the central methylene rather less efficiently than they supply the C_9 units. Some activity would be expected in the C_9 units, incorporated either <u>via</u> eventual participation of acetate, through pyruvate, in phenylalanine metabolism, or through degradation to CO_2. It would be surprising if such processes were more effective in acetate or malonate utilisation than the direct incorporation into the central methylene shown in Scheme 1. On the other hand, the pattern of labelling may be explained by the alternative scheme 3 (<u>cf.</u> ref. 4) which invokes polyketide extension of a cinnamate group with five acetate (malonate) units, and cyclisation of the hypothetical intermediate (2) as the source of the second aryl ring. In this route reduction as in (2) is required, either (3) or (4) being formed, and biosynthesis being completed by hydroxylation and methylation. Biosynthesis would thus be unsymmetrical, with different origins for the two C_9 parts. All the activity not in the central methylene is in this case confined to one C_9 unit, and not equally distributed between both. The small but distinct degree of scrambling which is apparent might be attributed to the activity of Krebs' cycle. The differences in distribution between acetate and malonate experiments can be accounted for if there are differences in pool size of intermediates. In addition, there may be



Scheme 3

developmental differences in individual plants.

Scheme 3 might involve (3) as the first symmetrical product. Hydroxylation and methylation then leads to curcumin (5) via (4). Both intermediates (3) and (4) can be isolated from C. longa, and we have confirmed the suggested structures[10] by a full spectroscopic study and by synthesis of (3).

Further work on the biosynthesis of curcumin is clearly required, and is in hand. Communication of these discordant experimental observations at this stage is however desirable to draw attention to the difficulties which arise in a ready acceptance of Scheme 1, and in view of the assumption that certain plant phenalenones[11] involve a fundamental construction from phenylalanine and tyrosine linked through the methyl of an acetate unit (a similar hypothesis to that made for curcumin at the start of our work). Acetate labelling positions have not been extracted in the work with phenalenones, and the possibility that phenylalanine and tyrosine are incorporated into the same C_9 unit has not been excluded.

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