Biosynthesis of Allylphenols in *Ocymum basilicum* L.

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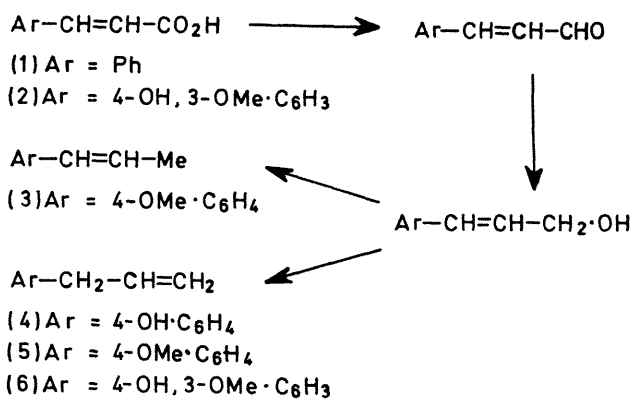
Summary Eugenol is synthesized in *O. basilicum* from L-phenylalanine *via* ferulic acid, whose side chain is converted into an allyl group with loss of the carboxy-group and incorporation of an 'extra' carbon atom (afforded, probably, by L-methionine).

IT is generally assumed[1,2] that allyl and propenyl groups attached to phenolic nuclei in many plants constituents, *e.g.*, anethole (**3**), chavicol (**4**), estragole (**5**), eugenol (**6**), *etc.*, originate from the cinnamic acid side chain through the reductive steps in the Scheme.

Rigorous proof for this hypothesis is still lacking; in fact, the only available precursor-incorporation data, from a study of the biosynthesis of anethole (**3**) in *Foeniculum vulgare*, do not appear conclusive.[3] We now emphasize that the foregoing assumption must be rejected, at least in the case of eugenol (**6**), chavicol (**4**), and estragole (**5**), because of the following evidence.



SCHEME

TABLE 1

Tracer experiments on Ocymum basilicum *L. 'Genovese'*

Expt.	Precursor[a]	Total activity (in μCi)	Specific activity (mCi/mM)	Duration of expt. (in h)	% Incorp. into eugenol (**6**)
1	L-[*U*-^{14}C]Phenylalanine	20	400	0·5	0·41
2	" "	20	400	1	0·12
3	" "	20	400	3	0·054
4	" "	20	400	24	0·034
5	[3-^{14}C]Cinnamic acid (**1**)	20	5	0·5	0·04
6	" "	20	5	1	0·11
7	[^{3}H]Ferulic acid (**2**)	5	0·05	1	0·06
8	L-[*U*-^{14}C]Tyrosine	20	400	0·5, 1, 24	$<10^{-4}$

[a] The method of Krotkov and Barker[5] was chosen for administering all the labelled precursors. Single sections of *O. basilicum* (35 g) used; at various times, these rapidly cut into small pieces and steam distilled. Eugenol was then isolated from the basil oil by preparative t.l.c. (Merck Silica Gel G; 0·5 mm; $CHCl_3$) and its identity and purity were checked by g.l.c.

Preliminary tracer experiments on *Ocymum basilicum* L 'Genovese' † showed that uniformly [^{14}C]labelled L-phenylalanine (but not L-tyrosine), [3-^{14}C]cinnamic acid (**1**) and non-specifically tritiated ferulic acid (**2**)‡ were incorporated into eugenol (**6**) (Table 1) This showed that the pathway L-phenylalanine → cinnamic acid → ferulic acid → eugenol occurred in this plant However, the finding that L-tyrosine is not incorporated into eugenol is in agreement with the lack of L-tyrosine ammonia-lyase in dicotyledonous plants [4] It is also noteworthy that maximum incorporation of L-phenylalanine into eugenol was observed after 0 5 h, thus indicating a very rapid rate of turnover for this substance

To study the transformation of the L-phenylalanine (or cinnamic acid) side chain into the allyl group in allylphenols, *O basilicum* ('Genovese' and 'Napoletano') was administered to DL-phenylalanines carrying a single ^{14}C-label at the three positions in the side chain All these DL-[^{14}C]phenylalanines were each mixed with non-specifically tritiated L-phenylalanine to provide doubly labelled specimens As shown in Table 2, the large rise in ^{3}H . ^{14}C ratios from the precursor to eugenol (**6**), estragole (**5**), and chavicol (**4**) in expts 3 and 4 suggests the loss of C-1 during the conversion of L-phenylalanine into allylphenols, which is likely to occur in the last stages of their biosynthesis

To investigate the origin of the 'extra' carbon atom required for the allyl side chain, we tested the incorporation of [^{14}C]methyl of L-methionine into chavicol (**4**), which contains no methoxy-groups Thus, an incorporation of *ca* 0 02% was observed after 1 h Introduction of the one-carbon atom unit into the side chain of cinnamic acid could, *a priori*, occur with or without rearrangement of the carbon skeleton and before, after, or simultaneously with the loss of the carboxy-group Work is in progress to elucidate the mechanisms involved

TABLE 2

Incorporation of DL *phenylalanine into allylphenols*

Expt	Labelling pattern[a]	^{3}H ^{14}C ratios[b] Precursor	Eugenol (**6**)	Estragole (**5**)	Chavicol (**4**)
1	[3 ^{14}C ^{3}H][c]	10 3	7 90		
2	[2 ^{14}C ^{3}H][c]	10 4	7 71		
3	[1-^{14}C ^{3}H][c]	9 05	200		
4	[1-^{14}C,^{3}H][d]	9 05		213	194

[a] Non-specifically tritiated L-phenylalanine was used as internal reference [b] See note (a) of Table 1 for administration and isolation of allylphenols Activities of eugenol were checked *via* its α naphthylurethane [c] *O basilicum* 'Genovese' [d] *O basilicum* 'Napoletano

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† *O basilicum* L (Labiatae) cultivated in Liguria, and named 'Genovese', was found to contain *ca* 0 08% of eugenol (15—20% relative to the steam-volatile oil) and 0 05% of ferulic acid By contrast, *O basilicum* cultivated in Campania and named 'Napoletano', showed estragole (0 1%) and chavicol (0 02%) as the only aromatic constituents of its essential oil (g l c –m.s)

‡ Labelled ferulic acid used in these experiments was isolated by preparative t l c (Merck Silica Gel G 1 mm, AcOEt–HCO_2H 6 4) from *O basilicum* 'Genovese' to which non specifically tritiated L-phenylalanine was fed (1 2% incorp) after addition of unlabelled ferulic acid this product was crystallized to constant specific activity

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