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BIOSYNTHESIS OF ANETHOLE IN *PIMPINELLA ANISUM* L.

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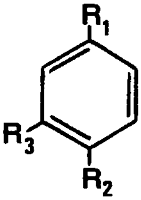
Until recently, the most plausible route for the biosynthesis of both allyl and propenylphenols seemed to be by a series of reductive steps from the corresponding cinnamic acids (Birch's hypothesis).[1] However, our finding that eugenol (I), estragole (II) and chavicol (III) are formed in *Ocimum basilicum* L.(Labiatae) from L-phenylalanine *via* oxygenated cinnamic acids with loss of the carboxy group and incorporation of an "extra" carbon atom into the side-chain[2] led to reject the above hypothesis for the origin of allylphenols, and, on reflection, raised new doubts about the biosynthesis of the propenyl isomers,[3] e.g. anethole (IV).

In this paper we report precursor-incorporation data which prove unequivocally that the phenylpropane skeleton of L-phenylalanine is incorporated in anethole (IV)[4] with retention of all carbon atoms, thus giving evidence for the *in vivo* occurrence of independent routes to allyl and propenylphenols.[5]



	R_1	R_2	R_3
(I)	$CH_2-CH=CH_2$	OH	OCH_3
(II)	$CH_2-CH=CH_2$	OCH_3	H
(III)	$CH_2-CH=CH_2$	OH	H
(IV)	$CH=CH-CH_3$	OCH_3	H
(V)	COOH	OCH_3	H

D,L-Phenylalanines labelled with ^{14}C at the side-chain in all three possible positions, each mixed with non-specifically tritiated D,L-phenylalanine, were fed to separate batches of ten flowering plants of *Pimpinella anisum* (Umbelliferae).[6] The plants of each batch were harvested after 20 days. Their aerial parts, when steam distilled, gave an oil which was added to radio-inactive anethole (*ca.* 50 mg) and chromatographed on silica gel column. Radio-active anethole, obtained by eluting with light petroleum (b.p. 40-70°)-benzene 8:2, was shown to be homogeneous (by g.l.c. and t.l.c.) and radiochemically pure (exhibiting constant specific activity and $^{3}H/^{14}C$ ratio after further chromatography).

TABLE

Incorporation of D,L-phenylalanine into anethole (IV)

Expt.	Labelling pattern	^{14}C Activity (in µCi)	% Incorp. of ^{14}C	^{3}H/^{14}C ratios		% Tritium loss (referred to ^{14}C)
				Precursor	Anethole	
1	[$3-^{14}C$, $G-^{3}H$]	100	0.132	5.77	3.31	42.6
2	[$2-^{14}C$, $G-^{3}H$]	50	0.141	17.01	9.38	44.8
3	[$1-^{14}C$, $G-^{3}H$]	50	0.124	13.32	7.88	40.9

The results shown in Table, reinforced by the lack of ^{14}C randomization (see below), demonstrate that the whole side-chain of phenylalanine is utilized to form the propenyl group of anethole, the constant loss of tritium, in all three experiments, being understandable considering the molecular modifications from precursor to product and the non-uniform labelling of the tritiated phenylalanine.

That no ^{14}C randomization occurred during the above experiments was shown by chemical degradation of anethole obtained by feeding [$3-^{14}C$, $G-^{3}H$]phenylalanine (expt. 1): permanganate oxidation (in aq. K_2CO_3) of such a radio-active anethole (diluted with inactive anethole: 315 mg; ^{14}C-activity: 5.9×10^{4} dpm mmol^{-1}) afforded anisic acid (V, 278 mg, crystallized from water to constant specific activity) which carried (on a molar basis) 94% of the original ^{14}C-activity.

This result, together with the data of Table, supports the early hypothesis[1] for the biosynthesis of propenylphenols.

REFERENCES

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2. L.Canonica, P.Manitto, D.Monti and M.Sanchez A., *Chem. Comm.* 1108 (1971).
3. cf. *Biosynthesis*, Specialist Periodical Report, vol.I, p.125, The Chemical Society, London, 1972.
4. An early biosynthetic study of anethole in *Foeniculum vulgare* (Umbelliferae) did not appear conclusive to this point: K.Kaneko, *Chem. and Pharm. Bull.* (Japan) **8**, 611, 875 (1960); **9**,108 (1961).
5. Bifurcation of the pathway leading to allyl and propenyl isomers occurs likely at cinnamic acids level. However, an interconversion (allyl ⟶ propenyl) *in vivo* cannot be excluded in certain plants containing both the isomers (cf. ref. 1b).
6. The aqueous solution of the labelled precursor was dropped by a syringe on the stems after scratching their surface with a blade.