A Mass-spectrometric Study of Biosynthesis: Conversion of Deutero-*m*-cresol into Patulin

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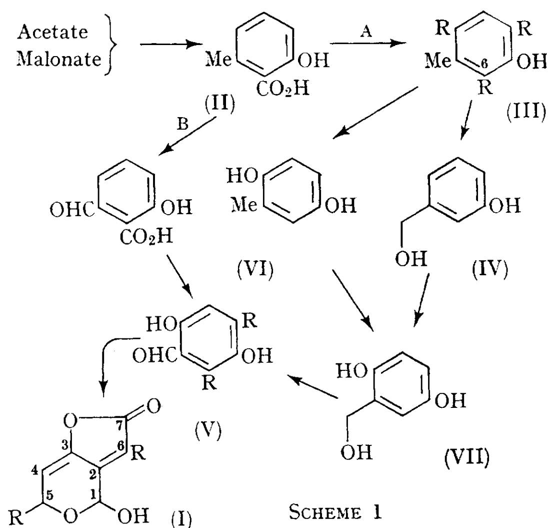
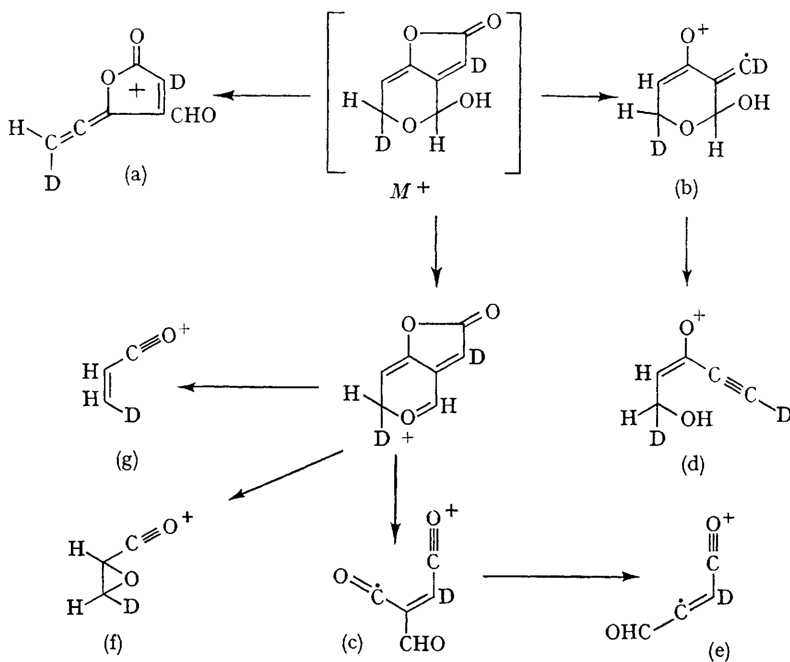
THE convenient and rapid direct mass-spectrometric mapping of the biosynthesis of indole alkaloids has recently been described.[1] The same technique has now been applied to the biosynthesis of patulin (I) in *Penicillim patulum*. Although conversion of 6-methylsalicyclic acid (II) into patulin (I) has been previously demonstrated,[2] the nature of other intermediates has remained obscure. The generally accepted pathway is as shown in Scheme 1, path B.

The discovery of several new phenolic metabolites of *P. patulum*, namely *m*-cresol,[3,4] (III; R = H) *m*-hydroxybenzyl alcohol[5] (IV) and toluquinol[4] (VI), suggested (III; R = H) as the most likely intermediate after 6-methylsalicylic acid (II). When [2,4,6-$^2H_3$]-3-methylphenol (III; R = D) was administered to *P. patulum* under normal culture conditions and the derived patulin examined in the mass spectrometer, the enrichment of the (M + 2) peak (156) corresponded to a 30% incorporation of (III; R = D) (after correction for the loss of one deuterium atom). Using a glucose-deficient medium the conversion of (III; R = D) into (I; R = D) was 57·9% ($M + 2/M = 1·4$). The mass spectrum of (I; R = D) disclosed seven principal fragment ions (a)—(g) (Table; Scheme 2)



SCHEME 1



SCHEME 2

in accord with the predicted labelling pattern. These assignments are supported by high-resolution mass measurements (Table) and by n.m.r. integration measurements which, for example, show the ratio of the C-5 protons in (I; R = H) to (I; R = D) to be 1·5/1·0.

TABLE

Relative abundance of (M + 2) *and* (M + 1) *peaks in deuteropatulin*

	Fragment		Ratio $(M+2)/M$	Ratio $(M+1)/M$
156	$M+2$		1·4	—
138	(a)		1·4	—
128	(b)		1·4	—
111	(c)	(111·0061)	—	1·3
99	(d)	(99·0392)	1·4	—
83	(e)	(83·0115)	—	1·5
72	(f)	(72·0197)	—	1·3
56	(g)	(56·0241)	—	1·3

Ratios were obtained in each case by comparison with the corresponding $(M-2)$ or $(M-1)$ peaks in unlabelled patulin.

Since deuterium from C-6 in (III; R = D) is retained at C-5 in patulin, ring fission of gentisaldehyde (V), a presumed intermediate, must be followed by a stereospecific reduction. In support of this step the deuteropatulin (I; R = D) was found to be optically active $[M]_{320}$ −122°; $[M]_{300}$ +3800°, whereas natural patulin is optically inactive in all accessible regions of the spectrum.

In addition, the toluquinol (VI), gentisyl alcohol (VII), and gentisaldehyde (V) obtained from this source showed a deuterium enrichment of the $(M+2)$ peak corresponding to respective incorporations of 71·0, 65·6, and 66·7% of (III; R = D). It is interesting to note that, in contrast to the observations of Witkop and others,[6] no intramolecular migration of deuterium during the course of the aromatic hydroxylation (III→ VI or IV → VII) is evident in our experiments as this would require the presence of an $(M+3)$ peak in the mass spectra of our products.

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[7] *Cf.* S. H. Pomeranz, *J. Biol. Chem.*, 1966, **241**, 161 and references cited, wherein mammalian tyrosinase is shown to hydroxylate tyrosine to Dopamine with *loss* of hydrogen *ortho* to the phenolic hydroxy-group. A similar effect has recently been observed in plants (A. R. Battersby) and in mammalian systems (J. Fishman [private communications]).