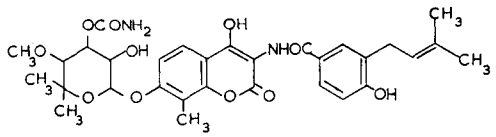
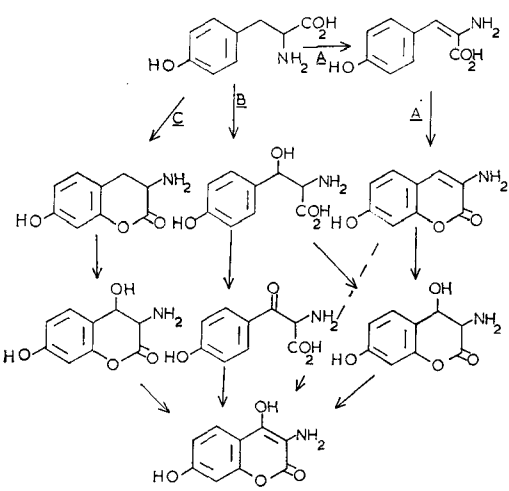
Investigations into the biosynthesis of novobiocin

Earlier Calvert, Spring & Stoker (1972) showed that the known biosynthetic pathways to 7-oxycoumarins were not involved in the biosynthesis of the aminocoumarin unit of novobiocin (I). We now wish to report investigations of other possible



(I)

biosynthetic pathways to this unit. There are at least three alternative pathways to this unit. These are: (A) Dehydrogenation of tyrosine to give 2-amino-4′-hydroxycinnamic acid, followed by direct cyclization to 3-amino-7-hydroxycoumarin(II), with subsequent hydration and oxidation leading to the aminocoumarin unit. Hydration of 2-amino-4′-hydroxycinnamic acid to 4′-hydroxyphenylserine(III) could precede cyclization and oxidation. (B) Hydroxylation of tyrosine at the β position, analogous to the conversion of 4′-aminophenylalanine to 4′-aminophenylserine (Vining, McGrath & others, 1968), to give III followed by oxidation and cyclization or vice versa could lead to the aminocoumarin unit. (C) Cyclization of tyrosine to give 3-amino-3,4-dihydro-7-hydroxycoumarin with subsequent introduction of the 4-hydroxy group as suggested in route (B) could also be a biosynthetic pathway to the aminocoumarin unit. The 8-methyl group is incorporated after the coumarin skelton is formed (Walton, Woodruff & McDaniel, 1962).



(II)

The pathways outlined above were investigated using techniques described previously (Calvert & others, 1972). Methyl-2-acetamido-4′-hydroxycinnamate-1,2-^{14}C(IV) was prepared by the method of Saul & Trikojus (1948). Attempts to prepare the free amino-acid were unsuccessful, it was expected that intracellular acylase and esterase enzymes would hydrolyse (IV) to the free amino-acid. Preparation of 3-amino-7-hydroxycoumarin was by the method of Rodighieo & Antonello (1958) followed by tritiation (Garnett, 1962), and DL-*threo*-4′hydroxyphenylserine-1,2-^{14}C by the method of Bolhofer (1954).

Details of specific activities of precursors, total activity fed and results of feeding experiments are given in Table 1. Data for the incorporation of L-tyrosine-U-^{14}C (Calvert & others, 1972) is given for comparison. Since yields of novobiocin were

Table 1. *Activity of compounds fed to cultures of* Streptomyces niveus *and results of feeding experiments.*

Precursor	S.A. of precursor	Activity Fed as μCi	S.A. of novobiocin	% Incorp. novobiocin	S.A. of amino-coumarin unit	% Incorp. into amino-coumarin unit	S.A. of benzoic acid unit	% Incorp. into benzoic acid unit
DL-Threo-4′-hydroxyphenylserine-1,2-^{14}C	0·142	10·15	2×10^{-4}	0·73	$1·3 \times 10^{-4}$	0·44	$0·03 \times 10^{-4}$	0·01
	0·142	10	$2·9 \times 10^{-4}$	0·95	$1·7 \times 10^{-4}$	0·57	$0·15 \times 10^{-4}$	0·04
Methyl-2-acetamido-4′-hydroxycinnamate-1,2-^{14}C	0·275	10·5	$0·9 \times 10^{-4}$	0·3	Insufficient activity to degrade			
3-Amino-7-hydroxy-coumarin G-^{3}H	1·36	69·3	$6·4 \times 10^{-4}$	0·42	$6·4 \times 10^{-4}$	0·42	0	0
	1·36	100	11×10^{-4}	0·6	$9·8 \times 10^{-4}$	0·53	0	0
L-Tyrosine-U-^{14}C	475	9·3	$1·7 \times 10^{-3}$	7·4	$9·5 \times 10^{-4}$	4·12	5×10^{-4}	2·12

S.A. = Specific activity expressed as mCi mM^{-1}
G = Generally labelled with ^{3}H

consistent throughout these investigations the values of percentage incorporation can be used as an indicator of a compound's ability to act as a precursor of novobiocin.

The intermediates from route A which were investigated, (II) and (IV), were both less efficient precursors of the aminocoumarin unit than tyrosine. Therefore it is unlikely that route A is a possible biosynthetic pathway to the aminocoumarin unit. These results also cast doubt on the involvement of a cyclization mechanism of the type suggested by Kenner, Bunton & others (1963). The poor incorporation of (III) compared to L-tyrosine-U-^{14}C, even allowing for the fact that the racemate was fed, suggests that route B is not involved in the biothsynthesis of the aminocoumarin unit. Therefore route C is the most probable pathway to the aminocoumarin unit of novobiocin. This route is at present under investigation.

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