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BIOSYNTHESIS OF ACTINOMYCIN: INCORPORATION OF [METHYL-$^2H_3$] METHIONINE, [^{3}H]KYNURENINE, AND [^{3}H]-3-HYDROXYKYNURENINE

R.B. Herbert

(Department of Organic Chemistry, The University of Leeds, Leeds LS2 9JT)

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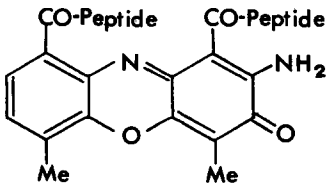
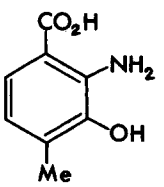
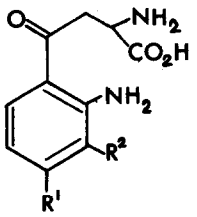
The actinomycins (1) are metabolites of Streptomyces antibioticus which find particular application biochemically in inhibiting DNA transcription.[1] The phenoxazinone skeleton of the antibiotics is known to arise from two molecules of tryptophan via 3-hydroxy-4-methylanthranilic acid (2),[2-4] methionine serving as the source of the aromatic methyl groups.[2,5] Depression of the incorporation of radioactive tryptophan into actinomycin by kynurenine (3)[4] and 3-hydroxy-4-methylkynurenine (5),[6] in washed cells of S. antibioticus, indicates that (3) and (5) may well be biosynthetic intermediates. In contrast 3-hydroxykynurenine (4) was found to have little or no effect on tryptophan incorporation[4,6] thus casting doubt on its participation in actinomycin biosynthesis.



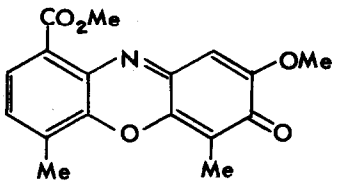
(1)



(2)

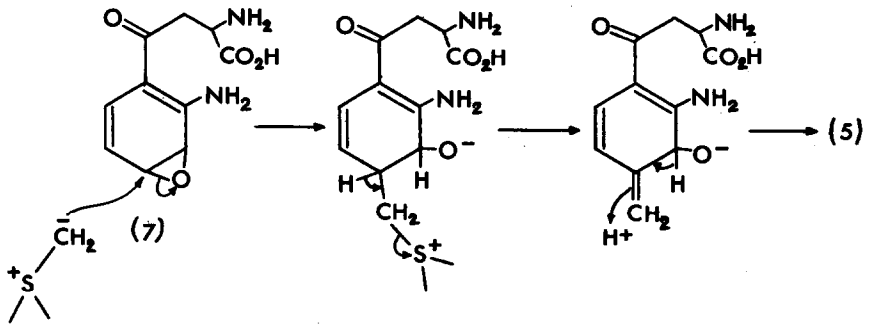


(3) $R^1=R^2=H$
(4) $R^1=H, R^2=OH$
(5) $R^1=Me, R^2=OH$



(6)

The normal metabolism of kynurenine (3) to 3-hydroxykynurenine (4) is mediated by a mixed-function oxidase and thus an arene oxide (as 7) is a likely intermediate.[7] The above observations suggested to us that perhaps actinomycin biosynthesis diverged from normal tryptophan metabolism by methylation of the oxide (7). Attack by the ylide of S-adenosylmethionine on (7) and subsequent proton shifts would afford 3-hydroxy-4-methylkynurenine (5) without the intermediacy of 3-hydroxykynurenine (see Scheme). Although this methylation mechanism is unprecedented, sulphur ylides have been proposed as intermediates in the biosynthesis of several naturally occurring compounds.[8]



Scheme

This hypothesis for actinomycin biosynthesis has been examined by feeding [methyl-$^{2}H_{3}$]methionine,[9] [^{3}H]-3-hydroxykynurenine,[10] and [^{3}H]kynurenine to S. antibioticus cultures over a 3 day period during antibiotic production. A 6% incorporation of the methionine into the phenoxazinone nucleus of actinomycin was recorded. The actinomycin obtained was converted into dimethylactinocinin (6)[11] and subjected to mass spectral analysis. It was clear from the spectra that only tri- and hexa-deuterio species were present. This eliminates an ylide methylation mechanism which requires loss of one deuterium atom on introduction of each methyl group (di- and tetra-deuteriomethylactinocinin) and provides incidental proof that both aromatic methyl groups originate from methionine.

[^{3}H]-3-Hydroxykynurenine and [^{3}H]kynurenine were found to be comparably effective precursors for actinomycin (1.6 and 2.8%, respectively). These results taken with those obtained with deuteriomethionine indicate that methylation follows formation of (4) and provides substance for the pathway: tryptophan ⟶ kynurenine (3) ⟶ 3-hydroxykynurenine (4) ⟶ actinomycin.

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