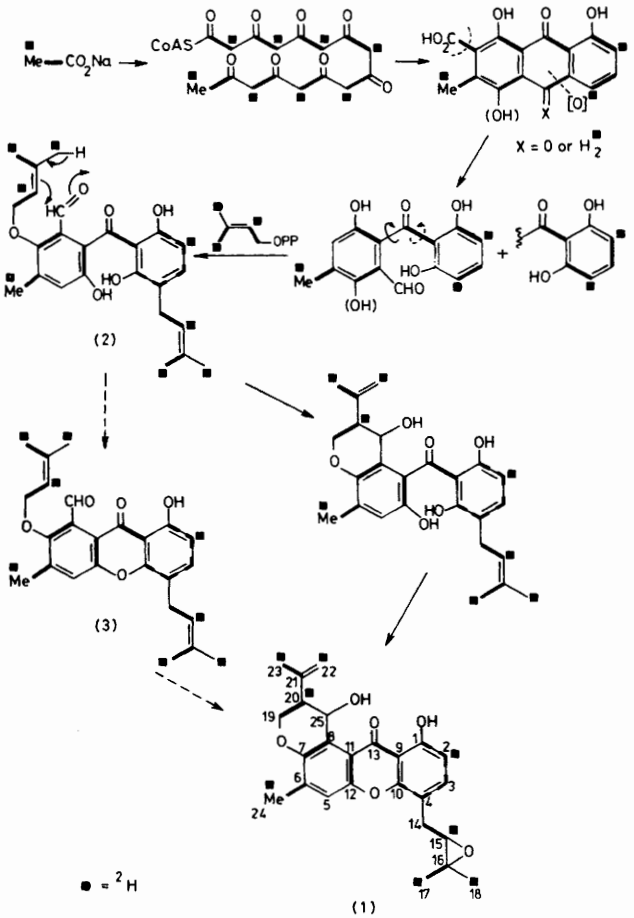
Biosynthesis of Tajixanthone in *Aspergillus variecolor*; Incorporation of [$^2H_3$]Acetate and [1,2-$^{13}C_2$]Acetate

By ESFANDIAR BARDSHIRI and THOMAS J. SIMPSON*
(*Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh* EH9 3JJ, *Scotland*)

Summary The results of ^{13}C and ^2H n.m.r. analyses of [$^2H_3$]- and [1,2-$^{13}C_2$]-acetate-enriched tajixanthone are reported, which indicate, *inter alia*, that ring cleavage of an anthraquinone, and not anthrone, precursor must precede *C*-prenylation, and that dihydropyran ring formation precedes xanthone ring formation during biosynthesis of tajixanthone.

Two major biosynthetic pathways are evident in *Aspergillus variecolor*, one leading to tajixanthone (**1**) and related mycelial pigments,[1] and the other to andibenin and related compounds isolated from the culture liquors.[2] Previous studies[3] have indicated that biosynthesis of tajixanthone occurs *via* an octaketide-derived anthrone or anthraquinone with the introduction of two prenyl units from 3,3-dimethylallylpyrophosphate (DMAPP) to give an *O*-prenyloxyaldehyde intermediate (**2**), which then undergoes an intramolecular 'ene' reaction to form the substituted dihydropyran ring, and cyclodehydration to form the xanthone system. Further indirect evidence in support of this pathway was given by a detailed study of the metabolites of a number of variant strains of *A. variecolor*,[1] but the sequence and mechanistic details of the required steps remained to be determined. We now report the results of incorporation studies with [1,2-$^{13}C_2$]- and [2-$^2H_3$]-acetate which, in conjunction with ^{13}C and ^2H n.m.r. data, allow some of these details to be elucidated.

The labelling patterns resulting from these incorporation studies† are summarised in the Scheme and the following conclusions can be drawn from the ^{13}C–^{13}C labelling pattern. (i) The acetate assembly pattern in the xanthone system is entirely consistent with an octaketide precursor folded as shown in the Scheme; *cf.* islandicin.[4] (ii) The randomisation of labelling in ring c means that ring c must have been symmetrical and free to rotate on the enzyme surface at some stage in the biosynthesis of tajixanthone. This means that ring cleavage of the carbocyclic precursor must precede introduction of the *C*-prenyl residue; *cf.* ravenelin.[5] (iii)



SCHEME

† The details of the ^{13}C and ^2H n.m.r. data will be published elsewhere.

C-Prenylation and epoxidation, in agreement with recent studies on echinulin[6] and flavoglaucin,[7] occurs with retention of configuration about the double bond of DMAPP. (iv) The stereospecificity of labelling in the dihydropyran ring suggests its formation from the *O*-prenylaldehyde moiety by a *concerted* 'ene' reaction. The transition state necessary for the observed 20,25-*trans* stereochemistry of (**1**) in a concerted reaction requires dihydropyran ring formation to occur before cyclodehydration to the xanthone system, as the transition state necessary for *trans* stereochemistry in an 'ene' reaction of the xanthone aldehyde (**3**) would have a highly unfavourable interaction between the aldehyde and xanthone carbonyls. Indeed, *in vitro* cyclisation of (**3**) gives the *cis* product.[8]

^{2}H N.m.r. spectroscopy has been successfully applied to the study of terpenoid biosynthesis but so far only to a few polyketide problems.[9] The apparent intermediacy of the aldehyde (**2**) in tajixanthone biosynthesis suggests that ring cleavage might occur at the anthrone rather than anthraquinone oxidation level, in which case H-25 would be derived from the hydrogen of acetate. As the 25-hydroxy-group of (**1**) is known to be resistant to reaction, *e.g.* oxidation, a ^{2}H-labelling study seemed appropriate. The ^{2}H n.m.r. spectrum of [$^2H_3$]acetate-enriched tajixanthone indicated the labelling pattern shown in the Scheme and permits the following conclusions. (i) There is no ^{2}H label on C-25; this implies cleavage of an anthraquinone rather than an anthrone intermediate. (ii) The absence of ^{2}H on C-5 indicates that decarboxylation of the octaketide precursor occurs *after* cyclisation and aromatisation. (iii) A differential incorporation of ^{2}H is apparent. The DMAPP-derived positions are enriched to a greater extent than the polyketide methyl which appears to be more highly enriched than the polyketide methylene positions. Indeed it is reassuring to see the presence of label on C-2 as, in two recent studies, ^{2}H was incorporated only into the acetyl coenzyme A-derived position and not those derived from malonyl coenzyme A.[10,11] (iv) There appears to be some loss of ^{2}H from the *E* methyl group relative to the *Z* methyl group of DMAPP (after allowing for the anticipated 2:3 ratio). This could be occurring from acetoacetyl coenzyme A, or could be due to dimethylallyl–isopentenyl pyrophosphate equilibration.

The above results allow the sequence of steps shown in the Scheme to be proposed for the biosynthesis of tajixanthone. Further studies to delineate the pathway are in progress and will be reported in due course.

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