Biosynthesis of Tajixanthone and Shamixanthone by *Aspergillus variecolor*: Incorporation of Oxygen-18 Gas

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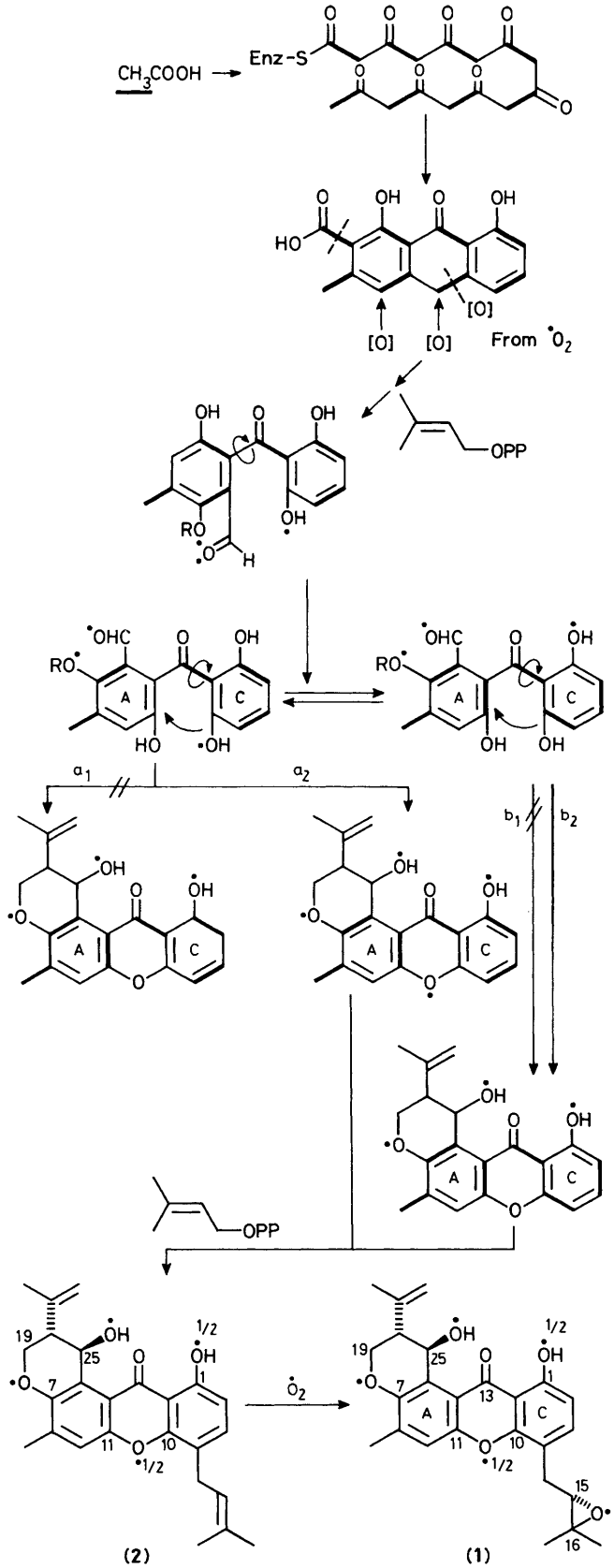
Mass spectral and ^{13}C n.m.r. analyses of tajixanthone (**1**) and shamixanthone (**2**) formed during growth of *Aspergillus variecolor* under atmospheres containing [$^{18}O_2$] oxygen gas showed incorporation of four and three ^{18}O labels per molecule of (**1**) and (**2**), respectively, and provided information about the mode of xanthone ring formation.

Mycelial pigments like tajixanthone (**1**) and shamixanthone (**2**)[1] as well as various meroterpenoids[2] illustrate how *Aspergillus* species can combine polyketide and terpenoid precursors to form secondary metabolites which have often undergone extensive oxidative elaboration. The isolation of a number of closely related xanthones[3—5] and ^{13}C and ^{2}H labelling studies[6,7] on tajixanthone strongly support the biosynthetic pathway outlined in Scheme 1. Carbon labelling results suggest that an acetate-derived octaketide precursor cyclizes to an anthrone which is hydroxylated, *O*-prenylated by dimethylallyl pyrophosphate, and oxidatively cleaved to a benzophenone derivative, either directly or after oxidation to an anthraquinone. Observation of two distinct carbon labelling patterns present in equal amounts in ring c of (**1**) implies

Table 1. ^{18}O Isotopically-shifted resonances in the ^{13}C n.m.r. spectra[a] of tajixanthone (**1**) and shamixanthone (**2**).

Carbon	δ (**1**)	Δδ (**1**) (× 100)	$^{16}O:^{18}O$ (**1**)[d]	δ (**2**)	Δδ (**2**) (× 100)	$^{16}O:^{18}O$ (**2**)[d]
13	184.0	2.7[b]				
1	160.4[c]	1.0	79:21	159.7	1.0[e]	
10	152.9[c]	2.3	80:20	152.8	2.5	71:29
11	152.0	2.4	77:23	152.2	2.3	73:27
7	149.5	1.5	61:39	149.4	1.6	53:47
19	64.5	2.3	66:34	64.5	2.5	55:45
15	63.24	3.3	64:36			
25	63.16	1.5	66:34	63.2	1.5	60:40
16	58.5	4.1	64:36			

[a] Spectra run at 100.6 and 90.6 MHz; for experimental conditions see ref. 9. [b] Enriched by sodium [1-^{13}C,$^{18}O_2$]acetate only; all others enriched by $^{18}O_2$. [c] These assignments were originally reversed in ref. 6. [d] Approximate (±5%) ratios from peak areas. [e] Not resolved completely.



R = $CH_2CH{=}CMe_2$
OPP = Pyrophosphate
Scheme 1

the intermediacy of a symmetrical dihydroxyphenyl moiety which is free to rotate prior to cyclization to a xanthone.[7] Since the detection of ^{18}O-induced isotope shifts in ^{13}C n.m.r.[8] has proved useful in determining the mode of xanthone ring formation in ravenelin[9] and sterigmatocystin,[10] we have studied the incorporation of $^{18}O_2$ gas into tajixanthone (**1**) and shamixanthone (**2**).

A fermentation of *Aspergillus variecolor*[1] in which the normal atmosphere was replaced with one containing $^{18}O_2$ gas (98.7% isotopic purity) gave tajixanthone (**1**), the mass spectrum of which showed the presence of four ^{18}O atoms per molecule. The 100.6 and 90.6 MHz ^{13}C n.m.r. spectra of a mixture of this and unlabelled material displayed isotopically-shifted resonances for eight of the nine oxygen-bearing carbons (Table 1). Only the carbonyl oxygen at C-13 remained completely unlabelled in this experiment. Within experimental error, the relative amount of ^{18}O incorporated at C-1 and at C-10 is half of that at the other labelled sites. Taken together with the mass spectral results, this shows that in a particular molecule of tajixanthone (**1**) either the oxygen at C-1 or the one at C-10 was labelled, but not both. This confirms the intermediacy and oxidative origin of a conformationally labile benzophenone which has an axis of symmetry in a dihydroxyphenyl ring. More importantly, the results demonstrate that xanthone ring closure must proceed almost exclusively by a Michael addition–elimination[11] process in which the ring C oxygen attacks the ring A carbon with ultimate loss of the ring A oxygen at C-11 (paths a_2 and b_2). Cyclization in the opposite sense with retention of the ring A oxygen (paths a_1 and b_1) is very minor if it occurs at all.

The presence of ^{18}O at C-25 and the previously reported loss of ^{2}H from acetate at that position[7] suggest oxidative cleavage of an anthraquinone rather than anthrone precursor. Mass spectral analysis of the molecular ion region of (**1**) obtained from a fermentation utilizing a mixture of $^{16}O_2$ and $^{18}O_2$ shows that each aerobically-derived oxygen atom is introduced separately by mono-oxygenation. Thus the involvement of dioxygenase-derived dioxetanes[12] or endoperoxides[13,14] which have been proposed as intermediates in the cleavage mechanism can be ruled out. Presumably cleavage occurs *via* a biological Baeyer-Villiger type oxidation[15] to give an intermediate lactone which can undergo direct reduction to the hemiacetal (*cf.* arugosin A/B[3]) and thence to the benzophenone.

In a separate experiment sodium [1-^{13}C,$^{18}O_2$]acetate (90% ^{18}O) was fed to cultures of *A. variecolor* grown in a normal atmosphere, and the resulting tajixanthone (**1**) was analysed by ^{13}C n.m.r. Unfortunately the incorporation level was too low to detect isotope shifts at any carbons except C-13, the C–O bond of which was thereby shown to be acetate-derived.

As expected, shamixanthone (**2**) isolated in the same experiment with $^{18}O_2$ showed, by mass spectral analysis, the incorporation of three ^{18}O atoms per molecule. Although the isotope shift in the ^{13}C n.m.r. of (**2**) at C-1 could not be completely resolved for accurate determination of the ^{16}O : ^{18}O ratio, the presence of ^{18}O at that site and the reduced ^{18}O content of the xanthone ring oxygen relative to other sites (Table 1) confirm the operation of the same biosynthetic pathway as that of tajixanthone (**1**). It is interesting to note that in ravenelin biosynthesis the same type of xanthone ring closure (paths a_2 and b_2) occurs with retention of the oxygens of a symmetrical dihydroxyphenyl moiety.[9] In contrast, retention of oxygen from the other ring and a single carbon labelling pattern during sterigmatocystin biosynthesis[10] suggest an oxidative coupling mechanism rather than addition–elimination for xanthone formation in that case.[16,17]

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