Biosynthesis of the Meroterpenoid, Austin, by *Aspergillus ustus*: Incorporation of $^{18}O_2$, Sodium [1-^{13}C,$^{18}O_2$]Acetate, and [*Me*-^{13}C,$^{2}H_3$]Methionine

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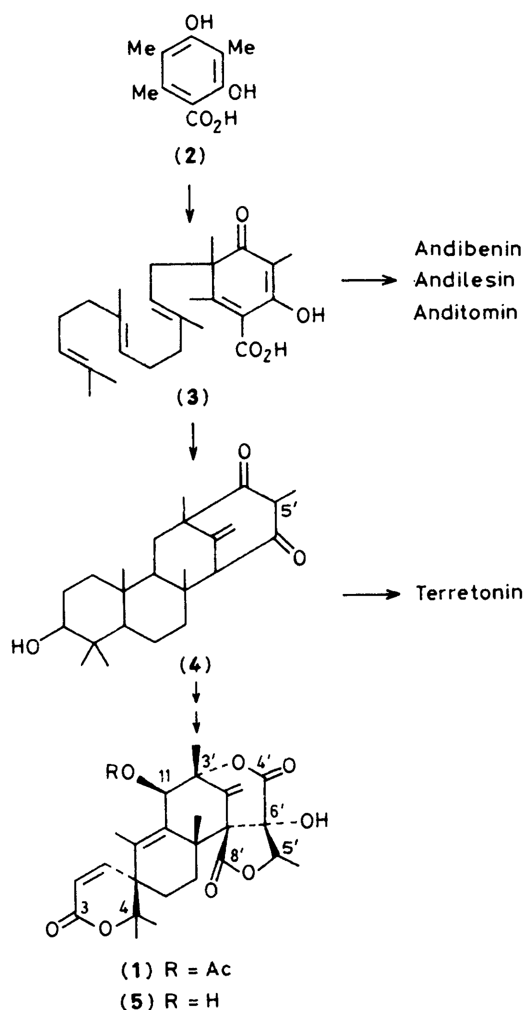
Mass spectral and ^{13}C n.m.r. analyses of austin (**1**) produced by fermentation of *Aspergillus ustus* in the presence of $^{18}O_2$ and [*Me*-^{13}C,$^{2}H_3$]methionine showed that all labelled methionine-derived hydrogens were retained, determined that five of nine oxygens were introduced by aerobic oxidation, and elucidated the general mechanism of late biosynthetic stages.

Recent studies show that andibenin B,[1] andilesin C,[2] anditomin,[2] austin (**1**),[3] and terretonin[3] may all be formed *via* a common biosynthetic precursor (**3**), which is the product of *C*-alkylation of dimethylorsellinic acid (**2**) by farnesyl pyrophosphate. Epoxidation of (**3**) followed by cyclisation could produce (**4**) which can serve as a common precursor to both austin (**1**) and terretonin (Scheme 1). The conversion of 3,5-dimethylorsellinic acid (**2**), a proven tetraketide precursor,[3] into austin (**1**) requires an unusually extensive modification of the aromatic ring. A number of chemically reasonable pathways from (**4**) to (**1**) (Scheme 2) may be distinguished because they require different origins for the various oxygen and hydrogen atoms. We now report ^{18}O and ^{2}H labelling studies which indicate that path a is correct and which provide information on other aspects of austin biosynthesis.



Scheme 1

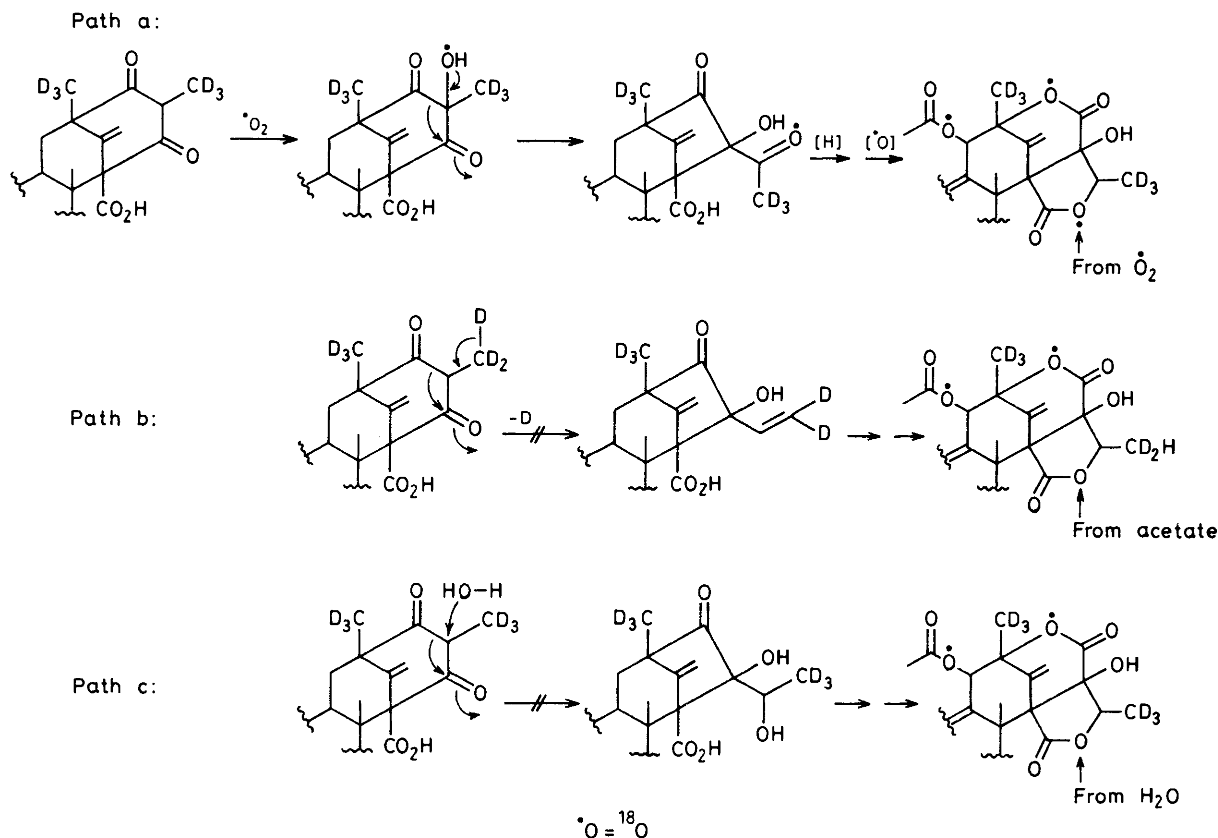
[*Me*-^{13}C,$^{2}H_3$]Methionine was added to cultures of *Aspergillus ustus* NRRL 6017 and the resulting labelled austin was analysed by fast atom bombardment (f.a.b.) mass spectrometry. Prominent $M+4$ and $M+8$ peaks demonstrated that the methionine-derived methyl groups of (**2**) are incorporated into austin (**1**) without loss of ^{2}H label. This result excludes the mechanism shown in path b.

A fermentation of *A. ustus* in which the normal atmosphere was replaced with one containing $^{18}O_2$ (96 atom %) after the onset of austin (**1**) production gave this metabolite labelled with up to five ^{18}O atoms per molecule, as determined by its mass spectrum. The positions of label incorporation were located by observation of ^{18}O isotope-induced shifts[4] in the 100.6 MHz proton noise decoupled ^{13}C n.m.r. spectrum of ^{18}O-enriched austin (**1**). An equal quantity of unlabelled austin had to be added to this sample as an internal reference because of the high level of ^{18}O incorporation and the small magnitude of such isotope shifts.[4] Although all four carbonyl carbons showed ^{18}O-isotopically shifted signals (Table 1), the magnitudes of these shifts for C-4′, C-8′, and the 11-acetate were consistent with presence of label only in the singly-bonded oxygen atoms. However for the C-3 resonance, three isotopically shifted signals appeared due to species having ^{18}O in (a) the singly-bonded oxygen, (b) the doubly-bonded oxygen, and mainly (c) both the singly and doubly-bonded oxygens.[5] Since the resonances corresponding to (a) and (b) probably result from residual $^{16}O_2$ in the fermentation, both oxygen atoms of the spiro-lactone are added by oxidative processes, in agreement with observations for andibenin B.[6]

Table 1. ^{18}O Isotopically shifted resonances observed in the 100.6 MHz ^{13}C n.m.r. spectrum of austin (**1**).[a]

Carbon	δ (p.p.m.)	Δδ (p.p.m. × 100)	Ratio ^{16}O : ^{18}O
8′	170.8	1.2	55:45
4′	170.2	1.3	57:43
MeCO	168.4	1.4	56:44
		3.8[b]	85:15
3	163.6	1.0, 3.7, 4.7	45:13:11:33
4	85.6	4.3	67:33
3′	84.4	3.8	60:40
6′	80.8	0.8	63:37
5′	78.9	3.1	66:34
11	74.9	2.7	67:33

[a] For experimental conditions see ref. 11. [b] Enriched by sodium [1-^{13}C,$^{18}O_2$]acetate; all others enriched by $^{18}O_2$.



Scheme 2

In the aliphatic region the resonances due to C-4, C-11, C-3′, and C-5′ all show isotope shifts which complement those observed in the carbonyl region, thereby accounting for all five ^{18}O atoms incorporated into austin. Interestingly, C-6′ also has a shifted signal (Table 1). The shift magnitude is too small for a tertiary alcohol,[7] and is due to a β-shift[8] from the ^{18}O atom attached to C-3′ or C-5′ or a double β-shift from both. This is the first observation of a β-shift in a biosynthetic study.

Since path c requires that the oxygen bridging C-5′ and C-8′ be derived from the medium, our results are consistent with an oxidation–reduction sequence like path a. The hydrogen,[3] carbon,[9] and oxygen labelling patterns suggest hydroxylation at C-5′ of (**4**), ring contraction *via* α-ketol rearrangement, reduction of the resulting 5′-keto function to an alcohol, and γ-lactone formation by attack of the 5′-hydroxy group on the 8′-carboxy function. Since no $^{18}O_2$-derived label is observed at the C-4′ carbonyl oxygen, this carbon cannot exist at any stage as a free carboxy group.

Introduction of the 11-acetoxy function of austin (**1**) probably occurs at a late biosynthetic stage by allylic hydroxylation and acetylation. Incorporation of sodium [1-^{13}C,$^{18}O_2$]-acetate into austin (**1**) followed by ^{13}C n.m.r. analysis showed that a large amount of ^{18}O label was present only at the double-bonded acetate oxygen in accord with its probable origin from acetyl coenzyme A. Incorporation of ^{18}O at other acetate-derived sites (C-4′, C-6′, and C-8′) was too low for definitive determination of isotope shifts. The isolation of austinol (**5**)[10] as a co-metabolite of austin (**1**) also supports the proposal that modification at C-11 is a late biosynthetic event.

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