Biosynthesis of the Meroterpenoid Metabolite, Andibenin B: Incorporation of Sodium [1-^{13}C,$^{18}O_2$]Acetate and $^{18}O_2$

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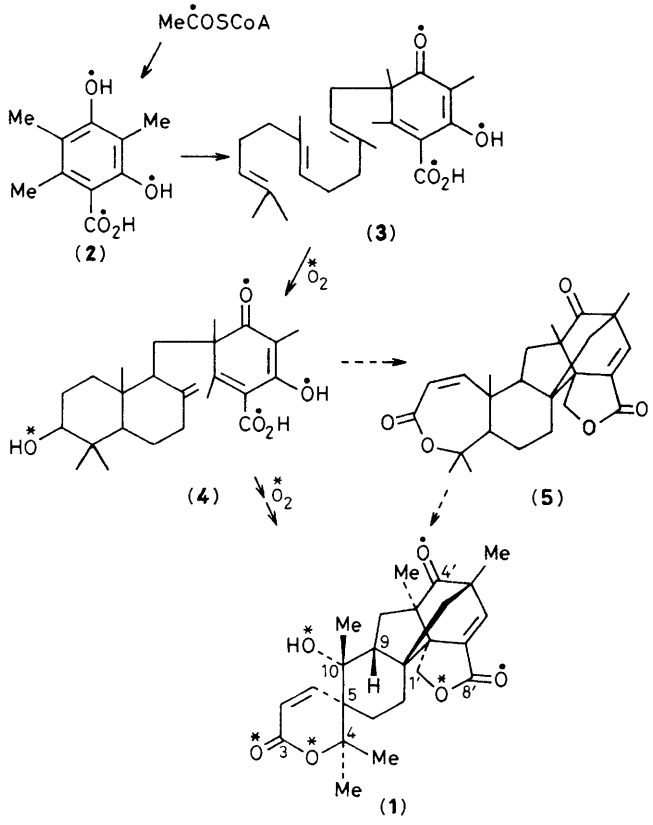
Incorporation of sodium [1-^{13}C,$^{18}O_2$]acetate and $^{18}O_2$ gas into andibenin B (**1**) by cultures of *Aspergillus variecolor* and observation of ^{18}O isotope-induced shifts in the ^{13}C n.m.r. spectra of the enriched metabolites establish the origins of all the oxygen atoms and provide mechanistic information on the biosynthetic pathway.

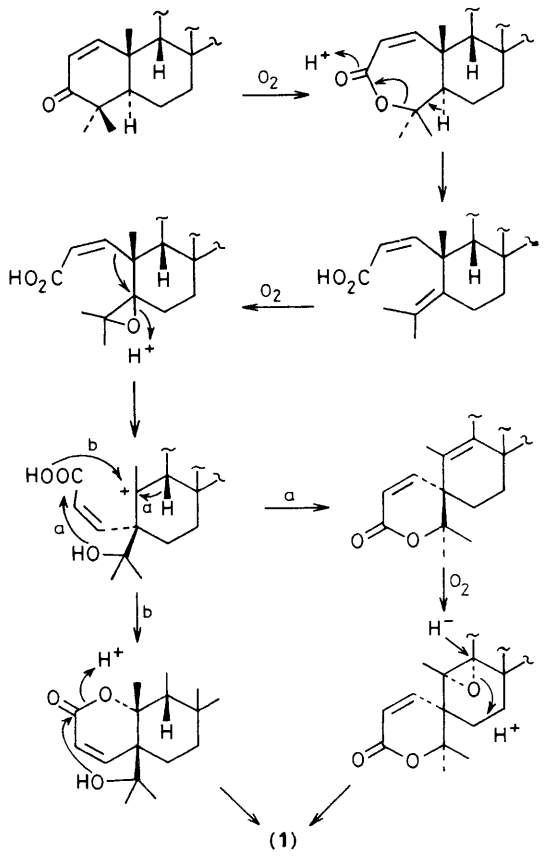
We have recently shown that andibenin B (**1**), a metabolite of *Aspergillus variecolor*, is a member of a group of biosynthetically related metabolites formed by a mixed polyketide–terpenoid pathway.[1,2] The key step is alkylation of 3,5-dimethylorsellinic acid (**2**), a bis-*C*-methylated tetraketide, with farnesyl pyrophosphate to give (**3**), which then cyclises to give product (**4**). Further condensations and oxidative modifications convert (**4**) into andibenin B (**1**). Since observation of isotope shifts induced in ^{13}C n.m.r. spectra by ^{18}O provides information on intermediate oxidation states and mechanisms,[3] we have incorporated sodium [1-^{13}C,$^{18}O_2$]acetate and $^{18}O_2$ gas into andibenin B (**1**) to study these modifications.

The proton noise-decoupled ^{13}C n.m.r. spectrum of (**1**) enriched by fermentation of *A. variecolor* with sodium [1-^{13}C,$^{18}O_2$]acetate shows isotopically shifted signals (Table 1) due to incorporation of acetate-derived oxygen into the C-4′ and C-8′ carbonyl groups. In a separate experiment, growth of the cultures under an $^{18}O_2$ atmosphere with unlabelled carbon sources produced andibenin B (**1**) whose ^{13}C n.m.r. spectrum demonstrated the origin of both oxygen atoms on C-3 and of the single-bonded oxygen atoms on C-4, C-10, C-1′, and C-8′ from oxidative processes. The appearance of oxygen label in the γ-lactone ring oxygen in the $^{18}O_2$ experiment suggests that the pathway proceeds by hydroxylation of the 6-methyl group of (**2**) followed by nucleophilic attack of this hydroxy group on the carboxy group. It remains to be established whether ring closure occurs before or after alkylation with farnesyl pyrophosphate.

In accord with earlier carbon labelling studies,[4] the present results show that the C-3 lactone function must be formed by a biological Baeyer–Villiger-type oxidation[5] of a corresponding ketone precursor. Generation of the spiro ring system involves a ring contraction which requires development of formal carbocation character at C-5. Similar ring contractions have been observed in steroid derivatives on acid treatment of either 4β,5β- or 5α,6α-epoxides.[6] Since the 10-hydroxy group of andibenin B (**1**) is derived from atmospheric oxygen, the rearrangement cannot terminate by capture of an intermediate C-10 carbocation by water. Instead, intramolecular attack by the carboxy group or an elimination–epoxidation–



Scheme 1



Scheme 2

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

Carbon	δ (p.p.m.)	Δδ (p.p.m. × 100)
4′	215.8	5.0[b]
8′	169.0	3.5[b]
		1.2[c]
3	165.5	4.7[c]
4	86.9	4.2[c]
10	79.3	3.2[c]
1′	70.4	2.9[c]

[a] For experimental conditions see ref. 9. [b] Enriched by sodium [1-^{13}C,$^{18}O_2$]acetate. [c] Enriched by $^{18}O_2$.

reduction sequence as shown in Scheme 2 is probably responsible for the hydroxy function. The closely related metabolite austin[2] contains the 9,10 double bond which would be present in the intermediate of the latter pathway. The co-occurrence of andilesin B (**5**) with andibenin B (**1**)[7] suggests that ring contraction follows lactone formation. Steroidal ε-lactones are known to undergo similar facile ring opening under acidic conditions.[8]

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