

Biosynthesis of Andibenin, a Novel Polyketide-terpenoid Metabolite of *Aspergillus varicolor*

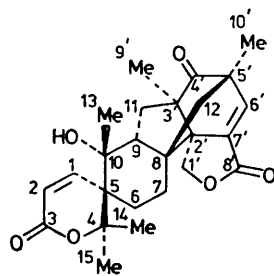
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Summary Incorporation of singly and doubly labelled [^{13}C]acetates and [*Me*- ^{13}C]methionine into andibenin by cultures of *A. varicolor* indicates its biosynthesis *via* alkylation of a bis-*C*-methylated, polyketide-derived aromatic precursor by farnesyl pyrophosphate.

ANDIBENIN, $\text{C}_{25}\text{H}_{30}\text{O}_6$, was recently isolated from static cultures of *Aspergillus varicolor* and its structure (1), determined by *X*-ray crystallography, suggested a biosynthetic pathway *via* a polyisoprenoid, possibly sesterterpenoid, precursor.¹ A preliminary report of ^{13}C -incorporation studies which indicate that andibenin is in fact of mixed polyketide-terpenoid origin is now presented.

Although the original yields of andibenin were too low for biosynthetic studies, it was found that an irradiation-induced mutant of *A. varicolor*, produced in connection with previous studies,² gave yields of andibenin of *ca.* 50 mg/l along with smaller amounts of several closely related compounds, including andilesin.³ Preliminary ^{14}C -studies indicated that the incorporation efficiency of acetate, though low, was sufficient for satisfactory ^{13}C -enrichments to be obtained. The ^{13}C -n.m.r. spectra of



(1)

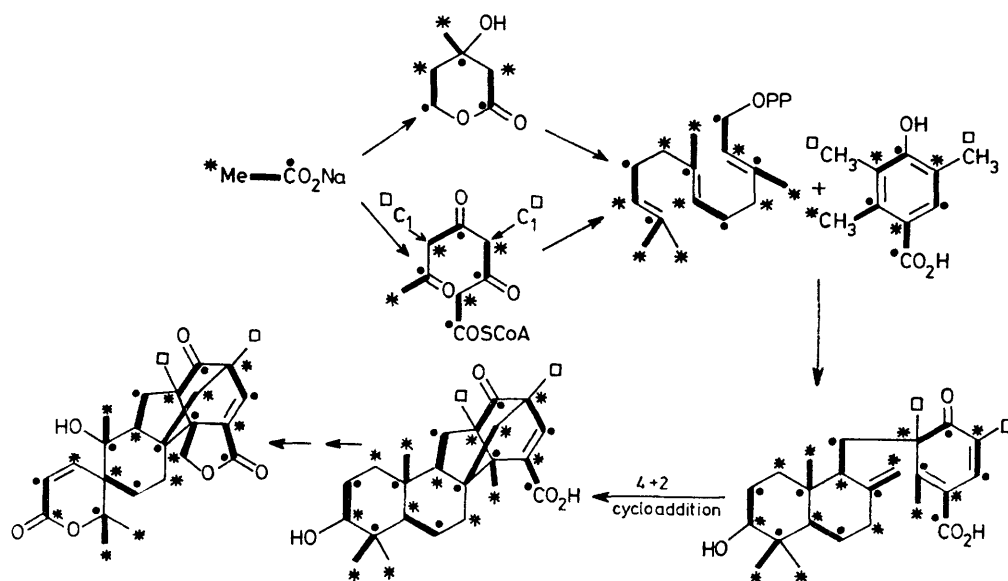
samples of andibenin, derived by incorporation of [$1\text{-}^{13}\text{C}$]-, [$2\text{-}^{13}\text{C}$]-, and [$1,2\text{-}^{13}\text{C}_2$]-acetates in cultures of *A. varicolor* showed the enrichments of individual atoms and ^{13}C - ^{13}C couplings summarised in the Table. The assignment of

TABLE. ^{13}C -Chemical Shifts (δ , relative to Me_4Si) of andibenin (1); coupling constants (Hz) of [$1,2\text{-}^{13}\text{C}_2$]acetate-enriched (1); and enrichments observed in [$1\text{-}^{13}\text{C}$]acetate (●), [$2\text{-}^{13}\text{C}$]acetate (*), and [*Me*- ^{13}C]methionine (□) enriched (1).

Carbon	δ /p.p.m.	1J (^{13}C - ^{13}C)	Enrichment ^d
1	151.8	—	*
2	118.3	65	●
3	164.2	66	*
4	85.3	41	●
5	46.7	34	*
6	35.1 ^a	34	●
7	27.8	—	*
8	58.5 ^b	36	●
9	52.7	35	*
10	77.2	38	●
11	28.9 ^a	34	●
12	55.6	37	*
13	23.6	39	*
14	24.5	40	*
15	27.0	—	*
1'	68.7	36	*
2'	48.9 ^b	36	●
3'	52.7	40	*
4'	213.9	40	●
5'	51.0	37	*
6'	140.9	37	●
7'	134.0	73	*
8'	167.4	73	●
9'	17.3 ^c	—	□
10'	17.2 ^c	—	□

a, b, c may be interchanged. ^d *ca.* 1 atom% for acetate, *ca.* 10 atom% for methionine.

the resonances in the ^{13}C -n.m.r. spectrum followed from standard shift data, off-resonance multiplets, and magnitudes of ^{13}C - ^{13}C couplings in the doubly labelled sample. Significantly the 9' and 10' methyl carbons were *not* enriched by acetate and so must originate from the C_1 -pool.



SCHEME

This was confirmed by feeding [*methyl*-¹³C]methionine, which resulted in high enrichments being observed at these two positions.

The results clearly indicate that andibenin is derived from two precursors, one of which is a sesquiterpene, and the other a tetraketide containing two *C*-methyl groups derived from methionine. A plausible route, involving the alkylation of the precursor phenol with farnesyl pyrophosphate is shown in the Scheme, an interesting feature being the proposed formal 4 + 2 cycloaddition which gives the correct carbon skeleton. Studies to determine the actual sequence of precursors and reactions are in progress.

Although several triprenyl phenols have been isolated from fungal sources,⁴ andibenin is unique in the position

of attachment of the sesquiterpene unit and the introduction of two alkyl substituents on the same carbon of the polyketide unit, and in having more than one carbon-carbon bond between the terpenoid and polyketide units. In addition the terpenoid spiro-lactone ring system has only been found in andibenin and in austin, a mycotoxin produced by *Aspergillus ustus*.⁵ A sesterterpenoid origin has been suggested for austin; however, a polyketide-terpenoid pathway analogous to that occurring in andibenin would also account for the biosynthesis of austin.

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