Biosynthesis of the Meroterpenoid Metabolite, Andibenin B: Aromatic Precursors

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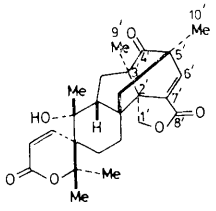
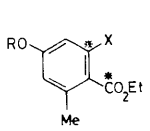
Summary ^{14}C- and ^{2}H-Labelling experiments, together with ^{2}H n.m.r. spectroscopy show that ethyl 2,4-dihydroxy-3,5,6-trimethylbenzoate (**4**) and ethyl 4-hydroxy-2,3,5-trimethylbenzoate (**5**), but not ethyl 2,4-dihydroxy-6-

methylbenzoate (**2**) or ethyl 4-hydroxy-2-methylbenzoate (**3**) are efficiently and specifically incorporated into andibenin B (**1**) by the fungus *Aspergillus variecolor*, and the biogenetic significance of these results is discussed.

ANDIBENIN B (**1**) is a major meroterpenoid metabolite produced by the fungus *A. variecolor*.[1,2] Incorporation experiments with [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-acetates, and [*methyl*-^{13}C]methionine have shown that this compound is derived from a sesquiterpene and a tetraketide, in which two *C*-methyl groups have been introduced from methionine.[3] Structural analysis of andibenin B and related co-metabolites suggested a number of possible tetraketide-derived phenolic carboxylic acids which might be directly involved on the biosynthetic pathway. To provide information on this point a series of ^{14}C-labelled compounds (**2**—**5**) and the trideuteriomethyl compound (**10**) have been synthesised and their incorporations studied.



(1)



(2) (R = H, X = OH)
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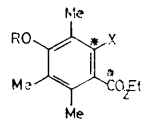
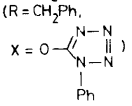
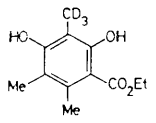


(4)
(5)
(8)
(9)

* = ^{14}C-label





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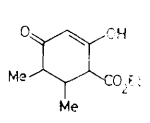

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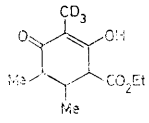

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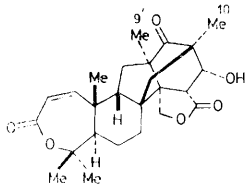
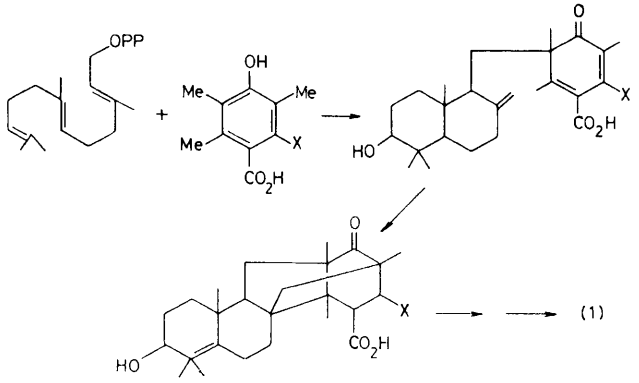


(13)

Ethyl [*carboxy*,2-$^{14}C_2$]-2,4-dihydroxy-6-methylbenzoate (**2**) was prepared by condensation of pent-3-en-2-one with diethyl [1-^{14}C]malonate in the presence of sodium ethoxide, followed by aromatisation of the ethyl dihydro-orsellinate with bromine to give ethyl dibromo-orsellinate, and subsequent hydrogenolysis.[4] Ethyl [*carboxy*,2-^{14}C]-2,4-dihydroxy-3,5,6-trimethylbenzoate (**4**) was similarly synthesised from 4-methylhex-4-en-3-one and diethyl [1-^{14}C]malonate, followed by dehydrogenation with bromine.†

The monohydric phenols (**3**) and (**5**) were synthesised from the respective dihydric phenols (**2**) and (**4**) by conversion into the monobenzyl ethers, (**6**) and (**8**) respectively, reaction of these with 5-chloro-1-phenyl-1*H*-tetrazole to give the 1′-phenyl-tetrazolyl derivatives, (**7**) and (**9**) respectively, and subsequent hydrogenolyses of these. This procedure for deoxygenation of phenols is an adaptation of a literature method[5] and gave satisfactory overall yields. The trideuteriomethyl compound (**10**) was prepared by monoalkylation, with trideuteriomethyl iodide and sodium ethoxide, of the cyclohexanedione (**11**) (enol form shown), prepared as above from 3-methylpent-2-en-4-one and diethyl malonate,[6] followed by aromatisation of the product (**12**) with bromine. Compound (**10**) showed only a singlet at δ 2·09 p.p.m. in the ^{2}H n.m.r. spectrum and the absence of a corresponding signal in the ^{1}H n.m.r. spectrum.

The aromatic compounds (**2**—**5**, and **10**) were fed to cultures of the *A. variecolor* mutant described previously,[3] and andibenin B was subsequently isolated. The incorporations of the ^{14}C-labelled compounds are summarised in the Table. The poor incorporations of ethyl orsellinate (**2**) and its deoxy-derivative (**3**), relative to those of the dimethyl derivatives (**4**) and (**5**), strongly suggests that only the latter two compounds, or more likely the corresponding carboxylic acids, are true biological intermediates. In the feeding experiment with the deuteriated compound (**10**) andilesin A (**13**) was isolated, in addition to andibenin B, and both were subjected to ^{2}H n.m.r. spectroscopy. The only observed signals were at δ 1·25 and 1·00 p.p.m. respectively, corresponding to those of the 10′-trideuteriomethyl groups in



X = H or OH

SCHEME

TABLE. Incorporations of ^{14}C-labelled aromatic precursors into andibenin B (**1**).[a]

Precursor	Specific activity μC mmol^{-1} (wt. fed/mg)	Specific activity of product μC mmol^{-1} × 10^2 (wt. isolated/mg)	Specific incorporation ratio/% × 10^2 [b]	Total incorporation ratio/% × 10^2 [c]
(**2**)	39·8 (80)	0·904 (43)	2·27	0·59
(**3**)	39·8 (80)	0·860 (89)	2·16	1·05
(**4**)	39·7 (83)	42·3 (173)	107	121
(**5**)	39·7 (85)	16·7 (176)	42·6	44·2

[a] Isolated by preparative layer chromatography and crystallised to constant activity. [b] Ratio of specific activities of metabolite and precursor × 100. [c] Ratio of total activities of isolated metabolite and fed precursor × 100.

† All new compounds have been fully characterised by elemental analysis and the usual range of physical methods. Details of their synthesis will be published elsewhere.

these compounds. Hence, it is clear that 2,4-dihydroxy-3,5,6-trimethylbenzoic acid is a specific precursor of this group of metabolites, as indicated in the Scheme.

It is known that C-9′ and C-10′ of andibenin-B (**1**) are derived from methionine, with C-1′ to C-8′ coming from a tetraketide. The differential between the incorporations of ethyl orsellinate (**2**) and the dimethyl derivative (**4**) provides clear evidence that biological *C*-methylation precedes aromatisation of the tetraketide, in contrast to the post-aromatic introduction of the sesquiterpene moiety.

Structural analysis of the andibenins and andilesins does not distinguish between monohydric and dihydric phenols as precursors. The feeding experiments described here, which had been designed to elucidate this point, are not decisive, as both the dihydric phenol (**4**) and its deoxy-derivative (**5**) are incorporated with comparable efficiency. The result suggests that both (**4**) and (**5**) can be utilised in the biosynthesis. It is intrinsically more attractive to consider that interconversion would follow introduction of the sesquiterpene residue, rather than direct biological interconversion of phenols (**4**) and (**5**).

(*Received*, 20*th August* 1981; *Com.* 1022.)

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