Biosynthesis of Ascochitine: Incorporation Studies with Advanced Precursors

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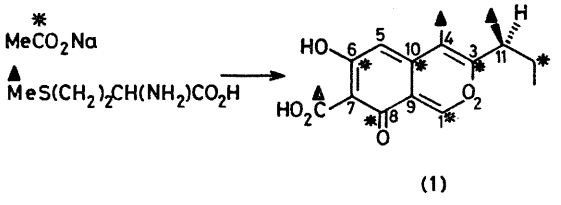
Summary Evidence is presented that the first step of the biosynthesis of ascochitine (**1**), a metabolite of *Ascochyta fabae* Speg., involves methylation of a hexaketide, followed by cyclization, reduction to the aldehyde, dehydration, and eventually oxidation of the methyl group at C(7) to a carboxy group.

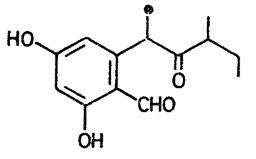
ASCOCHITINE (**1**),[1] a phytotoxic fungal metabolite of *Ascochyta fabae* Speg.[2] and *Ascochyta pisi* Lib.,[3] is derived from a single hexaketide chain, composed of head-to-tail acetate units, and three C_1 units introduced by *S*-adenosylmethionine. Incorporation of [1-^{13}C]acetate and [Me-^{13}C]methionine into (**1**) confirms this hypothesis (Scheme 1).[4]



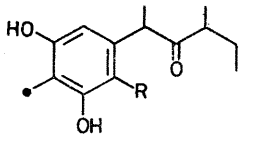
SCHEME 1

Detailed analysis of the ^{13}C–^{1}H long-range coupling constants allows the assignment of the *ortho*-quinone methide structure (**1**) to ascochitine.[4]

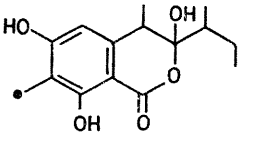
The most probable sequence of reactions involved in the biosynthesis of this phytotoxin, resulting from incorporation of the potential advanced precursors (**2**)—(**7**) is here described. Compounds (**2**)—(**7**) were obtained by alkylation of suitable synthetic intermediates with $^{14}CH_3I$.



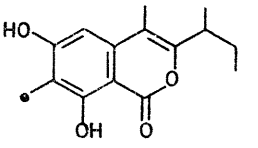
(2)



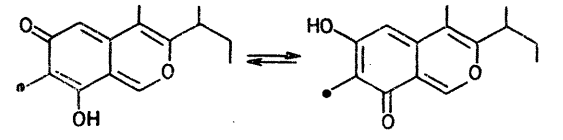
(3) R = CHO
(4) R = CO_2Me



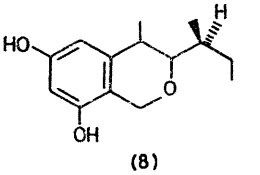
(5)



(6)



(7)



(8)

• = ^{14}C

These were added to cultures of *Ascochyta fabae* (3-day culture broths); 9 days after the addition, (**1**) was isolated as previously described.[2] The specificity of incorporation was tested by reduction of (**1**) to tetrahydroascochitine, and subsequent decarboxylation to compound (**8**). In agreement with analogous biosynthetic schemes,[5] the incorporation data (Table) exclude methylation of the aromatic nucleus as part of the biosynthetic pathway.

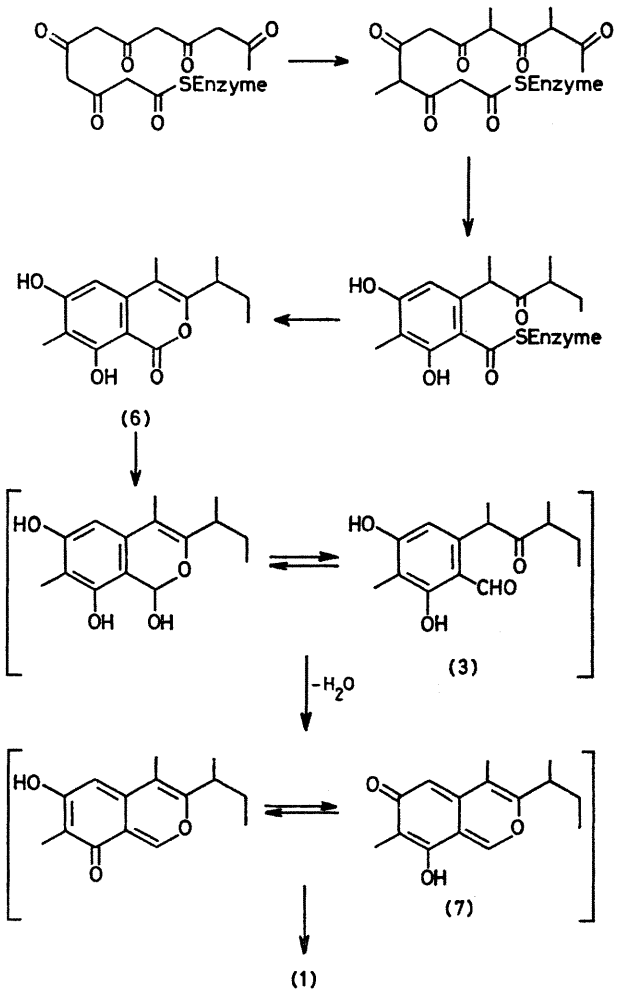
TABLE. Incorporation of the advanced precursors (**2**)—(**7**) into (**1**).

Expt.	Precursor	% Incorporation into (**1**)	% Recovery of label in (**8**)[a]
1	(**2**)	0·00	—
2	(**5**)	0·49	83
3	(**4**)	0·97	0
4	(**6**)	1·34	0
5	(**3**)	9·91	0
6	(**7**)	17·57	0

[a] % Recovery of label in CO_2 is complementary. Acid was added to the basic reaction mixture and the CO_2 thus liberated was quantitatively absorbed by Hyamine hydroxide—10 X (J. M. Passmann, N. S. Radin, and J. A. D. Cooper, *Analyt. Chem.*, 1956, **28**, 484) and counted.

The non-specific labelling of ascochitine derived from (**5**) indicates its degradation to acetate prior to incorporation (Table; expt. 2). The specific incorporation of the un-natural methyl ester (**4**) shows that the micro-organism can hydrolyse the ester with no formation of the lactol (**5**), and can apparently transform it into the enzyme-bound ester. At present the experimental data do not exclude the direct reduction of the enzyme-bound ester into the aldehyde (**3**). The most probable sequence of reactions and intermediates involved in ascochitine biosynthesis is shown in Scheme 2.

The optical rotatory power of ascochitine derived from (**3**) and (**7**) is lower than the normal value. Thus the enzymatic systems of *A. fabae* can transform intermediates characterized by an un-natural *R*-configuration at C(11) into ascochitine. The optical purity of ascochitine derived from (**3**) and (**7**) and the molar % incorporation of these intermediates were in good agreement.



SCHEME 2

We thank Professor H. Oku for kindly giving us the strain of *Ascochyta fabae*.

(*Received, 8th February* 1979; *Com.* 128.)

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[2] H. Oku and T. Nakanishi, *Phytopathology*, 1963, **53**, 1321.
[3] S. Bertini, *Ann. Stazione Chim.-agrar. sper. Roma*, 1956, **11**, 545.
[4] L. Colombo, C. Gennari, G. Severini Ricca, C. Scolastico, and F. Aragozzini, *J.C.S. Perkin I*, submitted for publication.
[5] T. Money, in 'Biosynthesis' (Specialist Periodical Report), ed. T. A. Geissman, The Chemical Society, London, 1973, vol. 2, pp. 183—212, and references therein.