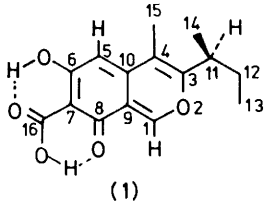
Biosynthetic Origin and Revised Structure of Ascochitine, a Phytotoxic Fungal Metabolite. Incorporation of [1-^{13}C]- and [1,2-$^{13}C_2$]-Acetates and [*Me*-^{13}C]Methionine

By **Lino Colombo, Cesare Gennari, Giuliana Severini Ricca,** and **Carlo Scolastico,*** Istituto di Chimica Organica dell'Università, Centro C.N.R. delle Sostanze Organiche Naturali, via Saldini 50, 20133 Milano, Italy

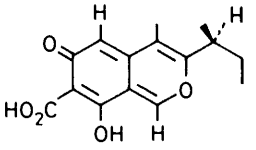
Fabrizio Aragozzini, Cattedra di Microbiologia Industriale dell'Università, via Celoria 2, 20133 Milano, Italy

A ^{13}C n.m.r. analysis of [1-^{13}C]- and [1,2-$^{13}C_2$]-acetate- and [*Me*-^{13}C]methionine-derived ascochitine confirms its origin from a single hexaketide chain and three C_1 units. An *ortho*-quinone-methide structure is assigned on the basis of the ^{13}C–^{1}H n.m.r. coupling patterns.

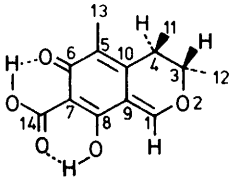
THE polyketide origin of ascochitine [1] (1), a phytotoxic fungal metabolite from culture filtrates of *Ascochyta pisi* Lib.[2] and *Ascochyta fabae* Speg.,[3] has been assumed by Turner,[4] on the basis of the analogy with the experimentally established biosynthesis of similar compounds, *e.g.* citrinin [5] (3). Our experiments with sodium



(1)

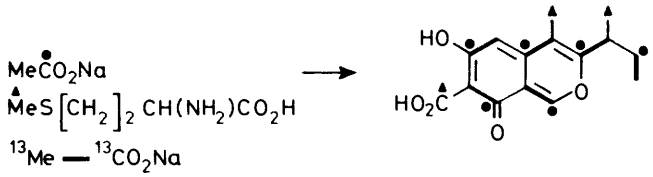


(2)



(3)

[1-^{13}C]- and [1,2-$^{13}C_2$]-acetates and [*Me*-^{13}C]methionine now confirm this hypothesis and show that the skeleton of ascochitine is derived from a single hexaketide chain, composed of head-to-tail acetate units, and possesses three C_1 units introduced by *S*-adenosylmethionine [6] (Scheme). This is confirmed by the ^{13}C chemical shifts,



SCHEME

which show the alternate distribution of the label in [1-^{13}C]acetate-derived (1), the presence of six pairs of coupled ^{13}C-satellites in [1,2-$^{13}C_2$]acetate-derived (1), and the presence of three labelled carbon atoms in [*Me*-^{13}C]methionine-derived (1).

A *para*-quinone-methide structure (2) had until now been assigned [1] to ascochitine and similar pyronoquinoid systems. A single-crystal *X*-ray diffraction study of citrinin (3) has shown that it is a *para*-quinone-methide.[7]

^{13}C–^{1}H Coupling patterns of (3) are in agreement with this structure (Table 1). In the proton-coupled ^{13}C n.m.r. spectrum unresolved multiplets originate from C-6 and from C-5, and are due to coupling to the three protons at C-13. Irradiation at the frequency of the signals due to these C-13 protons causes the multiplets due to C-5 and C-6 to change into singlets. Irradiation at the frequency of the 1-H and 8-OH signals causes the broad singlet due to C-8 to be enhanced in intensity; by irradiation at the frequency of the C-14-carboxy-proton signal, the signal from C-14 changes into a sharp singlet.

TABLE 1

^{13}C Chemical shifts (p.p.m.) and ^{13}C–^{1}H coupling constants (Hz) of citrinin

Carbon	Chemical shift	Multiplicity	*J* [coupled proton]
1	163.3	dd	200 [1-H]; 6 [3-H]
3	81.9	m	
4	34.5	m	
5	122.8	m	
6	183.6	m	
7	100.1	br s	
8	177.1	br s	
9	107.3	m	
10	139.5	m	
11	18.4		
12	18.2		
13	9.4		
14	174.4	br s	

In the proton-coupled ^{13}C n.m.r. spectrum of ascochitine (1) (Table 2), however, a double doublet originates from C-5, due to the coupling to 5-H {^{1}J[C-5–5-H] 168 Hz} and the hydrogen-bonded C-6-hydroxy-proton {^{3}J[C-5–6-OH] 6 Hz (*anti*)}. On deuterium exchange, or on irradiation at the frequency of the C-6-hydroxy-proton signal, the double doublet from C-5 changes into a simple doublet (owing to the loss of its coupling through three bonds), and the signal from C-6 is enhanced in intensity. By irradiation at the 5-H frequency the signal from C-6 changes into a doublet {^{2}J[C-6–6-OH] 3 Hz}. Irradiation at the frequency of the C-1 proton signal causes the signal from C(8) to change to a sharp singlet.

In this way analysis of the long-range coupling

constants [8] allows the assignment to ascochitine of the *ortho*-quinone-methide structure (1). The ^{13}C assignments have been confirmed by the observed ^{13}C–^{13}C couplings in the spectrum of ascochitine derived from [1,2-$^{13}C_2$]acetate (Table 2).

TABLE 2

^{13}C–Chemical shifts (p.p.m.), ^{13}C–^{1}H coupling constants (Hz), and ^{13}C–^{13}C coupling constants (Hz) of ascochitine

Carbon	Chemical shift	Multiplicity	*J* [coupled proton]	*J*(^{13}C–^{13}C) [a]
1	158.3 [b]	d	200 [1-H]	71.3
3	161.9 [b]	m [d]		52.2
4	117.4	m [d]		43.8
5	100.3	dd	168 [5-H]; 6 [6-OH]	64.8
6	179.1 [b]	br s [e]	3 [6-OH]	65.4
7	101.3	ddd [e]	6 [5-H]; 4 [6-OH] 4 [16-OH]	62.9
8	177.7 [b]	br s		63.6
9	115.0	dd	7 [5-H]; 7 [1-H]	71.2
10	141.4 [b]	m [d]		42.5
11	36.8			51.6
12	27.8 [b]			35.0
13	11.9			34.9
14	18.1 [c]			
15	12.3 [c]			
16	173.9 [c]	br s		

[a] ^{13}C–^{13}C Coupling constants in the proton noise decoupled ^{13}C n.m.r. spectrum of ascochitine derived from [1,2-$^{13}C_2$]-acetate. [b] Enhanced in intensity after incorporation of [1-^{13}C]acetate (average enrichment 2%). [c] Enhanced in intensity after incorporation of [Me-^{13}C]methionine (average enrichment 6%). [d] Unresolved multiplet. [e] Resolved by selective proton decoupling or by deuterium exchange.

EXPERIMENTAL

Incorporations of [1-^{13}C]acetate (90%), [1,2-$^{13}C_2$]acetate (90%), and [Me-^{13}C]methionine (90%) were achieved by addition of these compounds (0.33 mg ml^{-1}) to a 1-day culture broth; 9 days after the addition, isotope-enriched ascochitine was isolated as described previously.[3]

^{13}C N.m.r. spectra were recorded on a XL-100 Varian spectrometer at 25.2 MHz for *ca.* 0.1M-solutions in $CDCl_3$ using a heteronuclear lock. The spectra were recorded using broad band decoupling and an off-resonance technique was used to determine the substitution at carbon atoms. An alternately pulsed or gated decoupling method was also employed and selective proton decoupling experiments were performed using a low decoupling power and irradiation at the specific proton resonance frequency in order to make unequivocal assignments of several carbon frequencies. All chemical schifts are quoted as p.p.m. downfield from internal Me_4Si.

[9/392 *Received*, 12*th March*, 1979]

REFERENCES

[1] I. Iwai and H. Mishima, *Chem. and Ind.*, 1965, 186; M. N. Galbraith and W. B. Whalley, *J. Chem. Soc.* (*C*), 1971, 3557.
[2] S. Bertini, *Ann. Stazione chim.-agrar. sper. Roma*, 1956, **11**, 545.
[3] H. Oku and T. Nakanishi, *Phytopathology*, 1963, **53**, 1321.
[4] W. B. Turner, in ' Fungal Metabolites,' Academic Press, London, 1971, p. 137.
[5] R. F. Curtis, C. H. Hassal, and M. Nazar, *J. Chem. Soc.* (*C*), 1968, 85.
[6] F. Lederer, *Quart. Rev.*, 1969, **23**, 453.
[7] O. R. Rodig, M. Shiro, and Q. Fernando, *Chem. Comm.*, 1971, 1553.
[8] F. W. Wehrli, *J.C.S. Chem. Comm.*, 1975, 663; Ching-jer Chang, *J. Org. Chem.*, 1976, **41**, 1881; P. Äyräs and C. J. Widén, *Org. Magnetic Resonance*, 1978, **11**, 551.