Detection of C–C Bond Fission during the Biosynthesis of the Fungal Triprenylphenol Ascochlorin using [1,2-^{13}C]-Acetate

By MASATO TANABE* and KAZUO T. SUZUKI
(*Life Sciences Division, Stanford Research Institute, Menlo Park, California* 94025)

Summary Studies on ascochlorin biosynthesis with 1,2-^{13}C-acetate and Fourier transform ^{13}C-n.m.r. reveal that the C-14 methyl group migrates in the process.

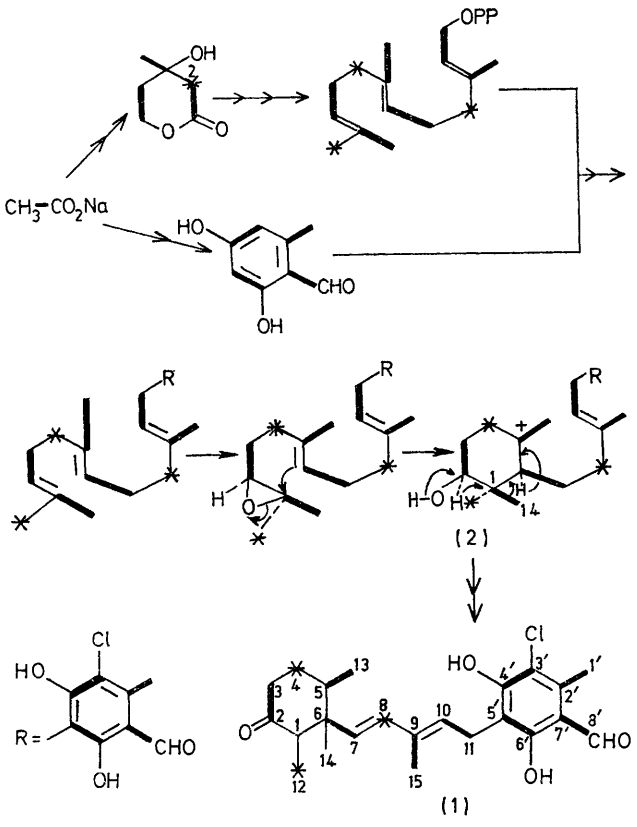
THE use of 90% enriched 1,2-^{13}C-acetate as a substrate in conjunction with Fourier transform (FT) ^{13}C-n.m.r. in elucidating pathways for the biosynthesis of fungal polyketides and terpenes has been established.[1] This method is now applied to the biosynthesis of the triprenylphenol, ascochlorin (**1**).[2]

TABLE. Carbon chemical shifts (δc)[a] and ^{13}C–^{13}C coupling constants of ascochlorin

Carbon chemical shifts					^{13}C–^{13}C Coupling constants/Hz
C-1	(d) 53·6				
C-2	(s) 212·8	C-3	(t)	41·6	37
C-4	(t) 31·1				
C-5	(d) 40·8	C-13	(q)	16·3[b]	37
C-6	(s) 48·5	C-7	(d)	135·6	45
C-8	(d) 133·2				
C-9	(s) 134·1	C-15	(q)	14·4	46
C-10	(d) 127·6	C-11	(t)	22·2	43
C-12	(q) 8·9[b]				
C-14	(q) 10·3[b]				
C-1′	(q) 12·6	C-2′	(s)	137·8	44
C-5′	(s) 113·8	C-4′	(s)	156·3	70
C-3′	(s) 113·2	C-6′	(s)	162·2	71
C-7′	(s) 111·7	C-8′	(d)	193·2	57

[a] Determination in deuteriochloroform on a Varian XL-100 at 25·2 MHz, in p.p.m. downfield from internal Me_4Si. [b] In the ^{1}H-n.m.r. spectrum, the C-12, C-13 and C-14 methyl protons appear at δ 0·71, 0·82, and 0·86 respectively. These carbons were assigned by selective proton decoupling at δ 0·70, 0·85 and 1·00 p.p.m.

Enriched (**1**) was isolated from cultures of *Nectria coccinea* containing 90% 1,2-^{13}C-acetate that was diluted three times with unlabelled acetate. In the proton noise decoupled FT-^{13}C-n.m.r. spectrum of labelled (**1**) 18 signals with ^{13}C–^{13}C coupling were detected, indicating that 9 acetate units were incorporated. The labelled sites appeared as triplets with characteristic ^{13}C–^{13}C coupling constants for the carbon satellites. These satellites appeared only when a doubly labelled acetate was incorporated and remained intact during the biosynthesis. The chemical shift and ^{13}C–^{13}C coupling assignments of the labelled metabolite were made by comparison with published results.[3] The assignments are given in the Table. The labelling results from this study are consistent with the biosynthetic pathway for ascochlorin as shown in the Scheme.



SCHEME

Detection of only 5 ^{13}C–^{13}C couplings rather than the usual 6 expected for the terpenoid derived part of (**1**) confirmed that a ^{13}C–^{13}C bond cleaved in the biosynthesis. The C-14–C-1 coupling was absent in the labelled metabolite and both the C-14 and C-1 signals appeared as enriched singlets. This result establishes that fission of the C-14–C-1 bond with stereospecific migration of the C-14 methyl

group to C-6 occurred probably in an intermediate of type (**2**).

The carbon signals at C-4, C-8, and C-12 appear as singlets with enhanced intensities. This result is consistent with cleavage of the C-1–C-2 bond in mevalonic acid during conversion into farnesyl pyrophosphate, and establishes the biosynthetic origin of these three carbons from C-2 of mevalonate. The 4 C–C couplings in the orsellinic aldehyde part of (**1**) confirm that its biosynthetic origin is through a polyketide and is the same as that of the fungal metabolite, orsellinic acid.[4]

We thank the U.S. Public Health Service for support of this work and Lewis W. Cary for the n.m.r. spectral data.

(*Received*, 19*th March* 1974; *Com.* 316.)

[1] H. Seto, T. Satō, and H. Yonehara, *J. Amer. Chem. Soc.*, 1973, **95**, 8461; H. Seto, L. W. Cary, and M. Tanabe, *J.C.S. Chem. Comm.*, 1973, 867.

[2] G. Tamura, S. Suzuki, A. Takatsuki, K. Ando, and K. Arima, *J. Antibiotics*, 1968, **21**, 539; G. A. Ellestad, R. H. Evans, and M. P. Kunstmann, *Tetrahedron*, 1969, **25**, 1323; S. Hayakawa, H. Minato, and K. Katagiri, *J. Antibiotics*, 1971, **24**, 653; D. C. Aldridge, A. Borrow, R. G. Foster, M. S. Large, H. Spencer, and W. B. Turner, *J.C.S. Perkin I*, 1972, 2136.

[3] L. F. Johnson and W. C. Jankowski, 'Carbon-13 N.m.r. Spectra,' Wiley-Interscience, New York, 1972; J. B. Stothers. 'Carbon-13 N.m.r. Spectroscopy,' Academic Press, New York, 1972; G. C. Levy and G. L. Nelson, 'Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley-Interscience, New York, 1972.

[4] W. B. Turner, 'Fungal Metabolites,' Academic Press, New York, 1971, p. 87.