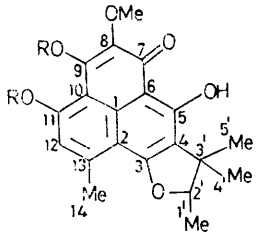
Biosynthesis of Deoxyherqueinone in *Penicillium herquei* from [^{13}C]Acetate and [^{13}C]Malonate. Assembly Pattern of Acetate into the Phenalenone Ring System

By THOMAS J. SIMPSON

(*Research School of Chemistry, Australian National University, P.O. Box 4, Canberra, A.C.T.* 2600, *Australia*)

Summary The ^{13}C-n.m.r. spectra of deoxyherqueinone (I) enriched with sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate, and diethyl [2-^{13}C]malonate by the fungus *Penicillium herquei* indicate formation of the phenalenone ring system from seven intact acetate units and the mode of folding of the precursor heptaketide chain; a clear acetate 'starter' effect is apparent in the [2-^{13}C]malonate-enriched ^{13}C-n.m.r. spectrum.

DEOXYHERQUEINONE (I) is one of a group of antibiotics based on the phenalenone ring system, produced by *Penicillium herquei*. ^{14}C-Tracer studies have indicated the polyketide origin of the nucleus and the mevalonate origin of the C_5 side chain;[1] the inter-relationships among this group of phenalenones have been elucidated by labelling



(I) R = H
(II) R = COMe

studies with *P. herquei*.[2] However, the nature of the intermediates leading from acetate and malonate to the phenalenone ring system, in common with the majority of phenolic polyketides, is unknown. Three alternate foldings of a single heptaketide chain, as well as multi-chain condensations, could account for the formation of the phenalenone ring system. With the advent of ^{13}C-n.m.r. spectroscopy in biosynthetic studies it has become possible to obtain direct information on these intermediates.[3]

TABLE. ^{13}C-Chemical shift (δ, relative to Me_4Si) of deoxyherqueinone diacetate (II); coupling constants (Hz) of [1,2-^{13}C]-acetate-enriched (II); and enrichments observed in [2-^{13}C]-malonate-enriched (II).

Carbon	δ/p.p.m.	^1J (^{13}C–^{13}C)	Enrichment[a]
1	125·2	55	0·9
2	112·4	65	2·8
3	165·5	64	0·9
4	119·8	68	2·9
5	173·1	68	0·9
6	107·6	60	2·9
7	173·9	59	1·0
8	141·3	85	2·5
9	142·9	85	1·0
10	110·9	56	2·7
11	148·2	68	1·0
12	121·6	68	3·3
13	142·1	42	1·3
14	23·4	42	1·9
1′	14·5	40	1·1
2′	91·0	40	1·6
3′	42·9	37	1·0
4′	20·3	—	1·7
5′	25·5	37	1·6
MeO	59·9	—	0·9
*C*H$_3$CO	20·7,21·1	—	1·3,1·3
CH$_3$*C*O	167·3,166·3	—	1·0,1·0

[a] See J. S. E. Holker, R. D. Lapper, and T. J. Simpson, *J.C.S. Perkin I*, 1974, 2135, for method of calculation.

Deoxyherqueinone was isolated, along with major amounts of what is believed to be herqueichrysin, a phenalenone of uncertain structure,[4] from the mycelium of *P. herquei* (C.M.I. 112950). The ^{13}C-n.m.r. spectrum of the diacetate (II) was assigned (Table) from literature values and detailed analysis of the fully proton-coupled spectrum. In order to facilitate comparison of incorporation efficiencies into the polyketide- and mevalonate-derived portions of the molecule, proton-noise-decoupled (p.n.d.) spectra were determined in the presence of 0·1 M [Cr(acac)$_3$] under GATED-2 decoupling conditions,[5] whereupon the very wide range of line intensities due to variable T_1 and N.O.E. factors was removed and almost integral intensities for all resonances in the natural abundance spectrum were obtained (Figure).

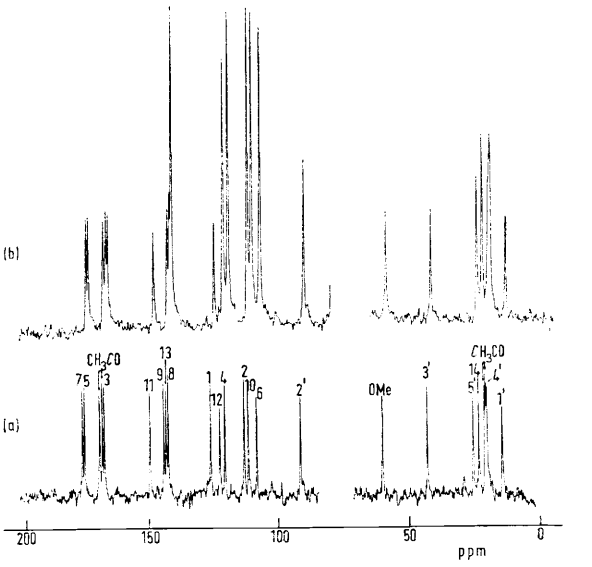
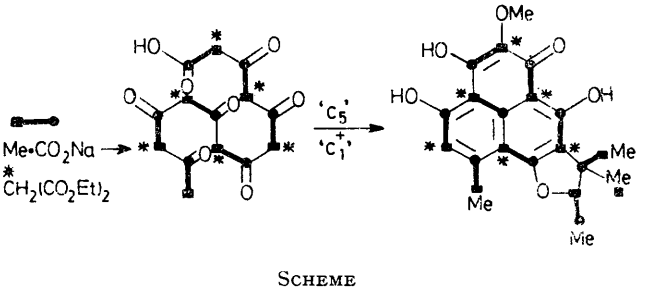


FIGURE. Proton-noise-decoupled, Fourier transform ^{13}C-n.m.r spectra of deoxyherqueinone diacetate (II), (a) at natural abundance, (b) enriched with [2-^{13}C]malonate, in 0·1 M [Cr(acac)$_3$] in $CDCl_3$ under GATED-2 decoupling.

The p.n.d. ^{13}C-n.m.r. spectra of the [1-^{13}C]- and [2-^{13}C]-acetate enriched samples showed the enhancements anticipated for the acetate origin of the molecule (Scheme). The observed enrichments were very high with *ca.* 9% excess ^{13}C-abundance at each labelled position, and with *equal* incorporation into the polyketide- and mevalonate-derived parts of the molecule. The [2-^{13}C]malonate-enriched spectrum showed high enrichment of six positions in the phenalenone nucleus: C(2), C(4), C(6), C(8), C(10), and C(12). The C(14) methyl, together with C(2′), C(4′), and C(5′) are also enriched but to less than half the extent (Table). Thus a clear acetate 'starter' effect is observed, indicating that the phenalenone ring system is formed from a single heptaketide chain.



SCHEME

The p.n.d. ^{13}C-n.m.r. spectrum of the [1,2-^{13}C]acetate-derived sample showed nine pairs of ^{13}C–^{13}C couplings,

indicating that C(14)–C(13), C(12)–C(11), C(10)–C(1), C(2)–C–(3), C(4)–C(5), C(6)–C(7), C(8)–C(9), C(5′)–C(3′), and C(2′)–C(1′) originate from intact acetate units. Thus the phenalenone ring system is formed by condensation of a heptaketide chain folded as shown (Scheme).

Other fungal phenalenones and their related metabolites[6] have been shown to be polyketide in origin and a similar assembly pattern of acetate units in their formation is likely. In the only previous biosynthetic study using [^{13}C] malonate, the malonate-derived carbon atoms in the 'ansa' chain of rifamycin S were enriched, with no significant enrichment of the acetoxy-substituent being observed.[7]

The author thanks Mrs. M. Anderson and Mrs. J. Rothschild for the microbiological work.

(*Received, 27th January* 1976; *Com.* 088.)

[1] R. Thomas, *Biochem. J.*, 1961, **78**, 807.
[2] A. P. Kriegler and R. Thomas, *Chem. Comm.*, 1971, 38.
[3] A. G. McInnes, D. G. Smith, J. A. Walter, L. C. Vining, and J. L. C. Wright, *J.C.S. Chem. Comm.*, 1975, 66; J. A. Gudgeon, J. S. E. Holker, and T. J. Simpson, *ibid.*, 1974, 636; P. S. Steyn, R. Vleggaar, P. L. Wessels, and D. B. Scott, *ibid.*, 1975, 193.
[4] N. Narasimhachari and L. C. Vining, *J. Antibiotics*, 1972, **25**, 155; D. D. Halton and G. A. Morrison, *Tetrahedron Letters*, 1975, 1443.
[5] O. A. Gansow, A. R. Burke, and G. N. La Mar, *J.C.S. Chem. Comm.*, 1972, 456; R. Freeman, H. D. W. Hill, and R. Kaptein, *J. Magnetic Resonance*, 1972, **7**, 327.
[6] R. Thomas, *Pure Appl. Chem.*, 1973, **34**, 515.
[7] A. Karlsson, G. Sartori, and R. J. White, *European J. Biochem.*, 1974, **47**, 251.