Carbon-13 Nuclear Magnetic Resonance Structural and Biosynthetic Studies on Deoxyherqueinone and Herqueichrysin, Phenalenone Metabolites of *Penicillium herquei*

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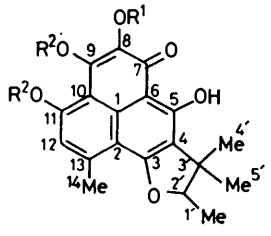
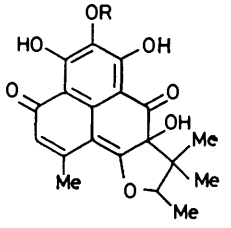
A detailed analysis of the $^{13}C-^{1}H$ couplings observed in the fully ^{1}H-coupled ^{13}C n.m.r. spectrum of deoxyherqueinone diacetate allow its structure to be defined as (3) and a full assignment of the ^{13}C n.m.r. spectrum to be made. The structure of herqueichrysin triacetate (8) is established by analysis of $^{13}C-^{1}H$ couplings in the fully ^{1}H-coupled, natural abundance ^{13}C n.m.r. spectrum, and $^{13}C-^{13}C$ couplings in the [1,2-$^{13}C_2$]acetate enriched ^{13}C n.m.r. spectrum. Further chemical and spectral studies allow structure (9) to be proposed for herqueichrysin. Incorporations of sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate, and diethyl [2-^{13}C]malonate indicate formation of the phenalenone ring system *via* a specific folding of a single heptaketide chain.

PENICILLIUM HERQUEI and *P. atrovenetum* produce a group of closely related antibiotics based on the phenalenone nucleus fused to a 1,2,2-trimethyldihydrofuran ring.[1] These are atrovenetin (1), deoxyherqueinone (2), herqueinone (4), and norherqueinone (5). ^{14}C Studies have shown that these phenalenones are derived *via* the acetate–polymalonate pathway, with methionine and mevalonate providing the carbons of the methoxy-group and the dihydrofuran ring respectively;[2] and labelling studies with *P. herquei* have provided evidence in favour of a sequential biosynthetic relationship between atrovenetin (1), deoxyherqueinone (2), and herqueinone (4); atrovenetin was incorporated into norherqueinone (5) but the latter was not converted into herqueinone (4) by *P. herquei*.[3] However, the nature of the intermediates

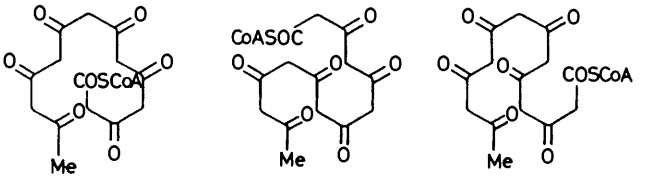


(1) $R^1 = R^2 = H$
(2) $R^1 = Me$, $R^2 = H$
(3) $R^1 = Me$, $R^2 = COCH_3$



(4) R = Me
(5) R = H

leading from acetate and malonate to the phenalenone ring system, in common with the majority of aromatic metabolites, is unknown. The ring system could be formed by any one of three alternate foldings of a single heptaketide chain as shown in Scheme 1, and in addition numerous multichain condensations are possible. With the advent of ^{13}C n.m.r. methods for biosynthetic studies, in particular the use of doubly labelled [^{13}C]acetate and analysis of the resultant $^{13}C-^{13}C$ coupling patterns it has become possible to obtain direct information on these intermediates.[4] *P. herquei* (CM1 112950) previously reported [5] to produce mainly atrovenetin (1) was chosen for this study. However in our hands, this strain produced deoxyherqueinone (2), along with major amounts of herqueichrysin, a phenalenone of uncertain structure,[6,7] only. Studies leading to the assembly



SCHEME 1 Alternative polyketide derivations of the phenalenone ring system

pattern of acetate units in the phenalenone ring system [8] and to structure (9) for herqueichrysin are now reported.

A prerequisite of any ^{13}C study is an unambiguous assignment of the ^{13}C n.m.r. spectrum. The metabolites were most conveniently isolated by acetylation and chromatography of the crude mycelial extracts. Thus, deoxyherqueinone was obtained as a diacetate, since chelation with the phenelenone carbonyl renders the *peri*-hydroxy very resistant to derivatisation. However, due to the tautomerism of the trihydroxyphenalenone system there are *a priori* four possible structures for this diacetate in which the ring carbonyl may be at C-5, C-7, C-9, or C-11. This uncertainty as to the precise structure makes assignment of the ^{13}C spectrum particularly difficult. However, a detailed analysis of the fully proton-coupled ^{13}C n.m.r. spectrum has enabled both the precise structure of the diacetate and unambiguous assignments to be determined.

In the proton-noise-decoupled (p.n.d.) ^{13}C n.m.r. spectrum determined under normal conditions a very wide range of resonance intensities is observed, the protonated carbons are the most intense and these are readily assigned from their chemical shifts, multiplicities in single frequency off-resonance decoupled (s.f.o.r.d.) spectra, and by specific proton decoupling experiments.[9] The remaining resonances of the phenalenone ring have been assigned by analysis of the low field portion of the fully proton-coupled ^{13}C n.m.r. spectrum in which most of the resonances exhibit long range $^{13}C-^{1}H$ couplings. The origin of all of these couplings has been established by use of deuterium exchange and, in particular, specific proton decoupling experiments using very low decoup-

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ling powers so that only the long-range coupling due to the proton being irradiated is removed with little or no perturbation of the remainder of the spectrum. The results of these experiments are summarised in Table 1.

TABLE 1

^{13}C Chemical shifts and $^{13}C-^{1}H$ couplings of deoxyherqueinone diacetate (3); $^{13}C-^{13}C$ coupling constants in [1,2-$^{13}C_2$]acetate enriched (3); and enrichments observed in [2-^{13}C]malonate enriched (3)

Carbon	δ [a]	$J^{13}C-^{1}H$	$^{1}J^{13}C-^{13}C$	Enrichment
1	125.2 s		55	0.9
2	112.4 dq	*C*–C–C–*H*,[c]	65	2.8
		C–C–*CH*$_3$ [b]		
3	165.5 s	*C*–O–C–*H* [f]	64	0.9
4	119.8 m	*C*–C–(*CH*$_3$)$_2$,[d]	68	2.9
		C–C–O–*H* [g]		
5	173.1 d	*C*–O–*H* [g]	68	0.9
6	107.6 d	*C*–C–O–*H* [g]	60	2.9
7	173.9 d	*C*–O–*H* [g]	59	1.0
8	141.3 q	*C*–O–*CH*$_3$ [e]	85	2.5
9	142.9 s		85	1.0
10	110.9 d	*C*–C–C–*H* [c]	56	2.7
11	148.2 d	*C*–C–*H* [c]	68	1.0
12	121.6 Dq	*C*–H,[c]	68	3.3
		C–C–*CH*$_3$ [b]		
13	142.1 q	*C*–*CH*$_3$ [b]	42	1.3
14	23.4 Q		42	1.9
1′	14.5 Q		40	1.1
2′	91.0 D		40	1.6
3′	42.9 S		37	1.0
4′	20.3 Q			1.7
5′	25.5 Q		37	1.6
MeO	59.9 Q			0.9
*C*H$_3$CO	20.7 Q			1.3
	21.1 Q			1.3
CH$_3$*C*O	167.3 q	*C*–*CH*$_3$		1.0
	166.3 q	*C*–*CH*$_3$		1.0

[a] Chemical shift relative to Me_4Si. Capital letters refer to the multiplicity resulting from directly bonded protons, and small letters to long range $^{13}C-^{1}H$ coupling. S = singlet, D = doublet, Q = quartet. [b] Removed on irradiation of 14-Me, τ 7.11. [c] Removed on irradiation of 12-H, τ 3.00. [d] Removed on irradiation of 4′, 5′-Me's, τ 8.5. [e] Removed on irradiation of OMe, τ 5.95. [f] Resonance enhanced on irradiation of 2′-H, τ 5.27. [g] Removed on addition of D_2O.

The two resonances at lowest field, 173.9 and 173.1 p.p.m. must be due to the carbonyl and chelated phenolic carbons and these appear as doublets, 2 and 5 Hz respectively, which on addition of D_2O sharpen to singlets. It is well established that in strongly hydrogen-bonded carbonyl systems, two-, three-, and four-bond $^{13}C-H$ couplings can be observed to the hydroxy-proton.[10] In addition, on deuterium exchange the 173.1 p.p.m. resonance displays an upfield shift of 0.6 p.p.m. This is large for a deuterium geminal isotope effect.[11] The other immediately noticeable change in the spectrum is the collapse of the doublet resonance at 107.6 p.p.m. to a singlet, and so this can be assigned to C-6, also displaying a three-bond coupling of 7 Hz to the phenolic proton. This coupling locates the chelated carbonyl system on carbons 5, 6, and 7. If it had been centred on carbons 9, 10, and 11, then C-11 would necessarily have appeared as a doublet on doublets, simplifying to a doublet on deuterium exchange, the remaining coupling being a three-bond coupling to the aromatic proton on C-12. Irradiation of the 12-proton at τ 3.00 (Table 2) in fact causes the doublet resonance at 110.9 p.p.m. (J 5 Hz) to sharpen to a singlet and so it is assigned to C-10. This irradiation also causes the doublet at 148.2 p.p.m. (J 3 Hz) to collapse to a singlet and so from its chemical shift it must be assigned to C-11, bearing an acetoxy group. Two-bond couplings of this type are not usually large enough to be resolved readily but they have been observed previously in phenolic acetates. The broad resonance at 112.4 p.p.m. also sharpens on irradiation of H-12, suggesting that it must be due to C-2 which should also exhibit a three-bond coupling to H-12. This is confirmed by irradiation of the 14-methyl protons at τ 7.11, whereupon the 112.4 p.p.m. resonance simplifies to a doublet (J 6 Hz) as expected, the residual coupling on removal of the three-bond coupling to the methyl protons being to H-12. This irradiation also causes the C-12 resonance which appears as a doublet (J 163 Hz, being the one-bond coupling to H-12) of quartets (J 6 Hz) to sharpen to a simple

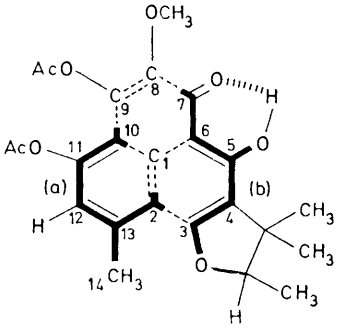
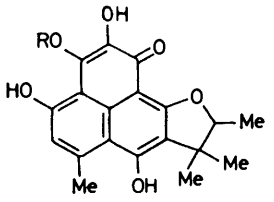


FIGURE 1 Partial structures in deoxyherqueinone diacetate (3) deduced from ^{13}C chemical shifts and $^{13}C-^{1}H$ coupling constants

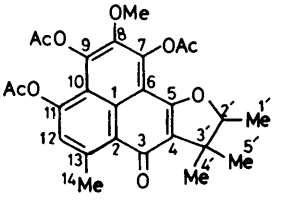
doublet due to removal of the three-bond coupling to the methyl protons; and more importantly the quartet at 142.1 p.p.m. (J 6 Hz) collapses to an intense singlet and so it is assigned to C-13. Irradiation of the methoxy-protons, τ 5.95, collapses the quartet (J 4 Hz) at 141.3 p.p.m. to a singlet allowing its assignment to C-8, its rather high field chemical shift being due to the presence of shielding oxygen substituents on both adjacent carbons. The crucial decoupling experiment involved irradiation of the *gem*-dimethyl protons of the dihydrofuran ring, τ 8.5, resulting in the collapse of the very broad resonance at 119.8 p.p.m. to a doublet (J 4 Hz) and so this resonance must be assigned to C-4, sharpening due to removal of the three-bond coupling to the methyl protons. The residual coupling is due to a three-bond coupling to the hydrogen-bonded hydroxy-proton since on repeating the irradiation in the presence of D_2O, the doublet splitting was also removed, and so C-5 must bear the phenolic hydroxy and thus C–7 the carbonyl function. Finally, irradiation of the dihydrofuranoid proton at τ 5.27 caused an approximately 50% increase in the intensity of the singlet resonance at 165.5 p.p.m. which was therefore assigned to C-3. The remaining very sharp singlets at 125.2 and 142.9 p.p.m. were assigned to C-1 and C-9 respectively, which showed

no long-range coupling as expected, there being no protons in a three-bond relationship to C-1 or C-9. These couplings allow the contiguous partial structures shown in Figure 1 to be deduced, and these define the precise structure of deoxyherqueinone diacetate as (3).

Two further major products were isolated from chromatography of the acetylated extract. ^{1}H N.m.r. spectroscopy (Table 2) allowed these to be identified as a monoacetate and a triacetate. On further acetylation the monoacetate was converted into the triacetate and it was apparent from its physical and spectral properties that the triacetate was identical to herqueichrysin triacetate. Herqueichrysin, a phenalenone isomeric with deoxyherqueinone (2), has recently been isolated from *P. herquei* [6,7] and on the basis of its physical and spectral properties, Vining *et al.* suggested structure (6).[6] This structure lacks *peri*-related carbonyl and hydroxy-functions as suggested by the formation of a triacetate in contrast to the diacetate obtained from deoxyherqueinone under identical conditions. On demethylation, demethylherqueichrysin (7) was obtained and this structure was subsequently confirmed by synthesis.[12] However the ready formation of a monoacetate is not consistent with structure (6) for herqueichrysin and the ^{1}H n.m.r. of the monoacetate does, in fact, show a strongly chelated hydroxy-proton at τ −6.8.



(6) R = Me
(7) R = H



(8)

^{13}C N.m.r. studies allow structure (8) to be deduced for herqueichrysin triacetate. The ^{13}C spectrum is summarised in Table 3. The aliphatic and protonated resonances were readily assigned as for deoxyherqueinone diacetate and examination of the fully proton-coupled spectrum allowed the remaining resonances to be assigned. The uncoupled resonance at lowest field, 182.5 p.p.m., must be assigned to the carbonyl carbon, less shielded than in deoxyherqueinone diacetate. Irradiation of the aromatic methyl protons caused the doublet of quartets due to C-12 centred on 127.0 p.p.m. (J 159 and 6 Hz) to sharpen to a simple doublet; the quartet (J 6 Hz) at 147.0 p.p.m. collapsed to an intense singlet, and the multiplet at 125.7 p.p.m. collapsed to a doublet (J 6 Hz) allowing their assignment to C-13 and C-2 respectively. Irradiation of the aromatic proton, τ 2.82, collapsed the doublets at 118.3 and 147.9 p.p.m. (J 5 and 3 Hz respectively) to singlets allowing their assignments to C-10 and C-11 respectively. Irradiation of the *gem*-dimethyl protons, τ 8.6, collapsed the broad resonance at 125.1 p.p.m. to a sharp singlet, and irradiation of the dihydrofuranoid proton, τ 5.47, caused a large increase in the intensity of the singlet resonance at 161.2 p.p.m. Irradiation of the methoxy-protons, τ 6.10, collapsed the quartet (J 4 Hz) at 143.4 p.p.m. to an intense singlet. The similarity of the chemical shift to the methoxylated carbon in deoxyherqueinone diacetate suggested a similar environment for it in herqueichrysin triacetate. Finally the singlet resonances at 126.4, 113.1, 144.3, and 142.1 p.p.m. can be assigned

TABLE 2

^{1}H N.m.r. chemical shifts (τ p.p.m.) of deoxyherqueinone and herqueichrysin and derivatives

Compound	12-H	14-CH_3	1′-CH_3	2′-H	4′,5′-$(CH_3)_2$	OCH_3	$COCH_3$
(2)	3.15	7.18	8.53	5.34	8.44, 8.68		
(3)	3.00	7.11	8.50	5.27	8.42, 8.63	5.95	7.58, 7.58
(8)	2.82	7.03	8.55	5.47	8.50, 8.72	6.10	7.59, 7.59, 7.60
(9)	3.30	7.11	8.44	5.20	8.36, 8.66	6.01	
(10)	3.25	7.12	8.54	5.43	8.44, 8.71	6.01	7.59

For $CDCl_3$ solutions, Me_4Si as internal standard.

TABLE 3

^{13}C Chemical shifts, ^{13}C–^{1}H couplings and ^{13}C–^{13}C couplings observed in herqueichrysin triacetate (8) and chemical shifts in herqueichrysin (9) and herqueichrysin monoacetate (10)

	(8)			(9)	(10)
Carbon	δ [a]	$J^{13}C-^1H$	$^1J^{13}C-^{13}C$	δ	δ
1	126.4s		57	126.6 s	126.7 s
2	125.7 dq	*C*–C–C–*H*,[c] C–C–CH_3 [b]	53	111.8 dq	111.9 dq
3	182.5 s		53	157.7 s	157.9 s
4	125.1 m	*C*–C–$(CH_3)_2$ [d]	66	118.7 m	120.1 m
5	161.2 s	*C*–O–C–*H* [f]	66	160.6 s	161.4 s
6	113.1 s		75	97.9 s	100.3 s
7	144.3 s		75	155.2 s	149.2 s
8	143.4 q	*C*–O–CH_3 [e]	82	132.9 q	140.6 q
9	142.1 s		82	177.0 s	178.2 s
10	118.3 d	*C*–C–C–*H* [c]	57	109.2 d	111.3 d
11	147.9 d	*C*–C–*H* [c]	73	171.4 s	172.2 s
12	127.0 D,q	*C*–*H*,[c] C–C–CH_3 [b]	73	120.1 D,q	119.5 D,q
13	147.0 q	*C*–CH_3 [b]	42	146.7 q	149.2 q
14	24.8 Q		42	24.9 Q	24.9 Q
1′	14.2 Q		40	14.0 Q	13.9 Q
2′	90.9 D		40	92.5 D	90.1 D
3′	43.9 S		37	43.1 S	43.1 S
4′	20.0 Q			21.0 Q	24.0 Q
5′	25.3 Q		37	25.5 Q	25.3 Q
OMe	61.9 Q			60.5 Q	60.6 Q
CH_3CO	20.6 Q, 20.6 Q, 20.9 Q				21.0 Q
CH_3CO	168.7 q 167.9 q 168.3 q	*C*–CH_3			168.8 S

[a] See footnote to Table 1. [b] Removed on irradiation of 14-Me, τ 7.03. [c] Removed on irradiation of 12-H, τ 2.82. [d] Removed on irradiation of 4′, 5′-Me's, τ 8.6. [e] Removed on irradiation of O-Me, τ 6.10. [f] Enhanced on irradiation of 2′-H, τ 5.47.

from their chemical shifts, to C-1, C-6, and the two remaining acetylated phenolic carbons. This data allows the partial structure shown in Figure 2(a) to be deduced. Incorporation of [2-^{13}C]acetate indicated that the methoxy-bearing carbon and the carbon resonating at 125.1 p.p.m. were derived from the methyl carbon of acetate and final evidence for structure (8) was provided by the spectrum of the tracetate enriched from [1,2-$^{13}C_2$]acetate. This showed that C-2 which has been definitely assigned is coupled to the carbonyl carbon which must, therefore, be placed at C-3; also C-6 is coupled to the resonance at 144.3 p.p.m. and the methoxy-bearing carbon is coupled to the remaining acetylated phenolic carbon at 142.1 p.p.m. as shown in Figure 2(b), thus defining structure (8) for herqueichrysin triacetate.

Herqueichrysin itself was readily obtained by saponification of the triacetate and proved identical in all respects to an authentic specimen. The precise structures of herqueichrysin and the monoacetate are more difficult to define. Both show strong absorptions in their i.r. spectra at 3 600 cm^{-1} characteristic of non-bonded hydroxy-group. The ^{1}H n.m.r. of the monoacetate shows the presence of a chelated hydroxy-proton, τ −6.84, and a non-bonded hydroxy, *ca.* τ 4.1. This chelated carbonyl system can only be accommodated by structure (10). The alternative structure (11) with

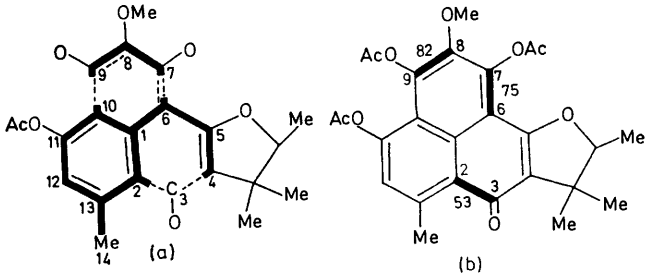
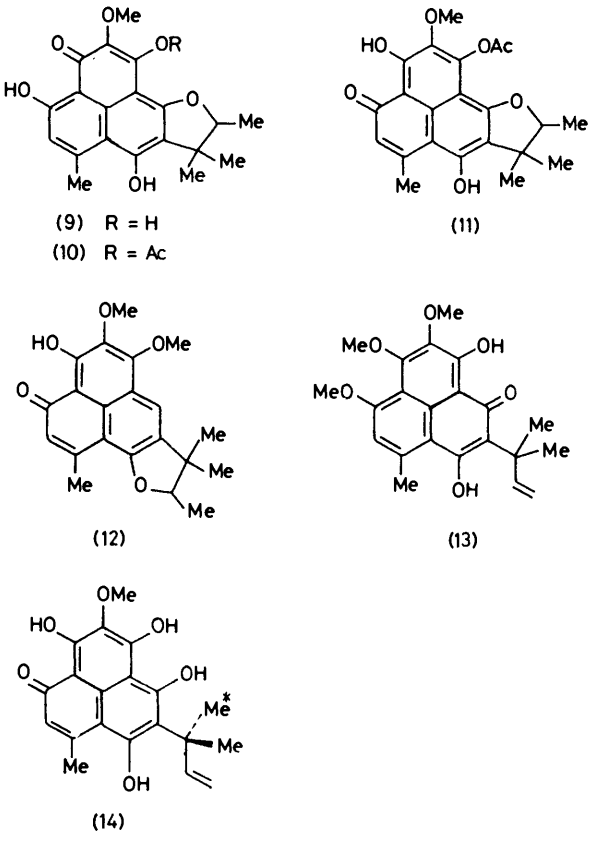


FIGURE 2 Partial structures in herqueichrysin triacetate (8) deduced from (a) ^{13}C—^{1}H couplings and ^{13}C chemical shifts, and (b) ^{13}C—^{13}C couplings

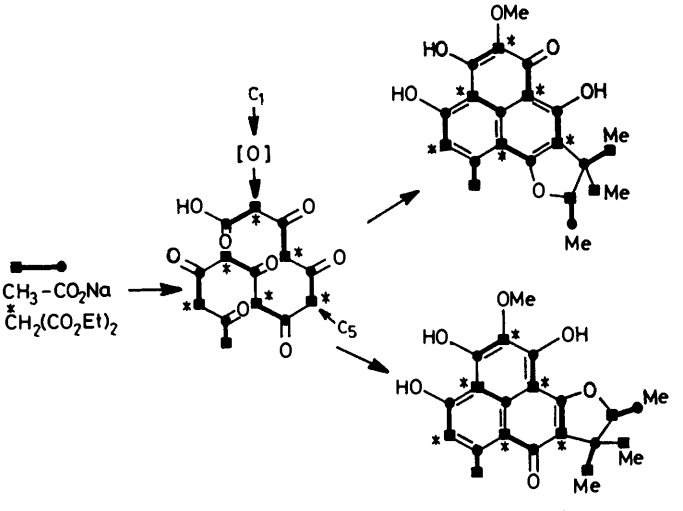
the carbonyl at C-11 is considered to be unlikely as the 12-proton resonates at τ 3.30 in herqueichrysin in contrast to compound (12) where the proton α to the carbonyl resonates at τ 2.97 and shows an allylic coupling (1.2 Hz) to the methyl protons.[13] No chelated hydroxy-proton is apparent in the ^{1}H n.m.r. spectrum of herqueichrysin itself but this can be attributed to the increased acidity of herqueichrysin [6] causing more rapid exchange to occur. Thus structure (9) is proposed for herqueichrysin itself and the ^{13}C n.m.r. spectra of her queichrysin and its monoacetate (Table 3) are in full agreement with these proposed structures.

Structure (9) satisfactorily accounts for the acetylation behaviour of herqueichrysin. The unhindered hydroxy group at C-7 reacts readily to give the monoacetate (10). It has been shown that in compound (13) the strongly chelated 7-hydroxy-group is preferentially alkylated as a result of the severe steric hindrance imposed on the 3-hydroxy-group; thus, in the case of herqueichrysin acetylation of the 11-hydroxy-function must be followed by tautomerism of the carbonyl to



C-3 to give a 9-hydroxy-group which will readily acetylate.

Preliminary experiments with [^{14}C]acetate indicated that acetate was efficiently incorporated into the phenalenone metabolites. Thus on feeding [1-^{13}C]- and [2-



SCHEME 2 Incorporation pattern of acetate and malonate into deoxyherqueinone and herqueichrysin

^{13}C]-acetate the p.n.d. ^{13}C n.m.r. spectra showed the enhancements anticipated for the acetate origin of the

metabolites (Scheme 2). In order to facilitate comparison of incorporation efficiencies into the polyketide and mevalonate derived portions of the molecules, p.n.d. spectra were determined in the presence of 0.1M-Cr(acac)$_3$[14] under Gated-2 decoupling conditions,[15] whereupon the very wide range of line intensities due to variable T_1 and nuclear Overhauser factors was removed and essentially integral intensities for all resonances in the natural abundance spectra were obtained. The enhancements observed in the spectra of the enriched samples were 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 molecules indicating full equilibration of the added [^{13}C]-acetate with the endogenous metabolic pools of acetate and mevalonate. The incorporation of [2-^{13}C]malonate

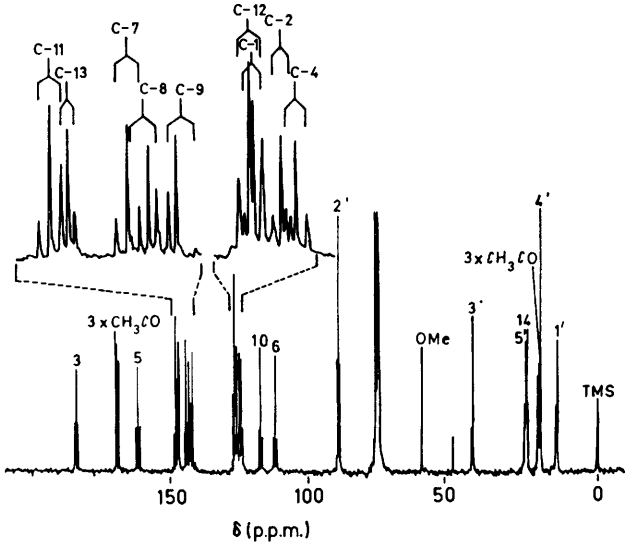


FIGURE 3 ^{13}C N.m.r. spectrum of [1,2-$^{13}C_2$]acetate enriched herqueichrysin triacetate determined at 67.89 MHz

is of more interest. As shown in Table 1 the overall enrichment was lower than with acetate. The values in Table 1 were calculated by multiple determination of both natural abundance and enriched spectra, which were averaged after normalisation [16] to overcome the variation in resonance intensities arising from digitisation and transformation of data. 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. Partial decarboxylation of the [2-^{13}C]malonate results in the C-14 methyl together with C-2', C-4', and C-5' also being enriched but to less than half the extent. Thus a clear acetate ' starter ' effect is observed, indicating that the phenalenone ring system is formed from a single heptaketide chain. The equal enrichment of C-14 and the mevalonate derived carbons 2', 4', and 5' from [2-^{13}C]malonate is consistent with the incorporation of malonate into mevalonate only after decarboxylation to acetate.

The p.n.d. ^{13}C n.m.r. spectra of the [1,2-$^{13}C_2$]acetate enriched samples showed nine pairs of ^{13}C–^{13}C couplings, indicating that carbons 14 and 13, 12 and 11, 10 and 1, 2 and 3, 4 and 5, 6 and 7, 8 and 9, 5' and 3', and 2' and 1' originate from intact acetate units. Due to severe overlap of the coupled ^{13}C signals and second-order ^{13}C–^{13}C couplings arising from the similarity of chemical shift of some of the coupled carbons, the ^{13}C n.m.r. spectra determined at the normal operating frequency of 15.04 MHz were extremely difficult to interpret. However on redetermining the spectra at 67.89 MHz the higher dispersion allowed the couplings to be resolved (see Figure 3). Thus the phenalenone ring system is formed by condensation of a heptaketide chain folded as shown in Scheme 2.

Herqueichrysin is the only member of the herqueinone group of metabolites to display the alternative orientation of the fused dihydrofuranoid ring. Their co-occurrence suggests that deoxyherqueinone and herqueichrysin are formed from a common prenylated phenalenone precursor, *e.g.* (14) which can cyclise to either of the adjacent phenolic hydroxy-groups. The ^{13}C n.m.r. data clearly show that in both herqueichrysin and deoxyherqueinone, enriched from [1,2-$^{13}C_2$]acetate, it is C-5' *trans* to C-1' which is coupled to C-3' (C-4' is not coupled as it arises from C-2 of mevalonic acid from which C-1 is lost in the formation of dimethylallyl pyrophosphate, the presumed precursor of the dihydrofuranoid ring). Thus both deoxyherqueinone and herqueichrysin must have the same configuration at C-2'.

Other fungal phenalenones and their related metabolites are polyketide in origin [1] and a similar assembly pattern of acetate units in their formation is likely.

EXPERIMENTAL

M.p.s were taken with a Kofler hot-stage microscope. Unless otherwise stated i.r. spectra were measured for solutions in chloroform, u.v. spectra in methanol, and ^{1}H n.m.r. spectra at 100 MHz, in deuteriochloroform with tetramethylsilane as internal reference. Mass spectra were recorded at 70 eV with an A.E.I. MS9 high-resolution spectrometer. Optical rotations were measured for solutions in chloroform at room temperature with an ETL-NPL automatic polarimeter. ^{13}C N.m.r. spectra were obtained for samples in acid-free deuteriochloroform with tetramethylsilane as internal reference. Proton-noise-decoupled spectra, single frequency off-resonance decoupled spectra, and fully proton-coupled spectra were determined on a JEOL JNM FX60 spectrometer operating at 15.04 MHz. Specific decoupling experiments were determined on 2500 Hz sweep widths using a single ^{1}H irradiating frequency of 16 dB. The spectra of [1,2-$^{13}C_2$]acetate enriched samples were determined on a Bruker WH-270 spectrometer operating at 67.89 MHz. Trisacetylacetonatochromium, Cr(acac)$_3$, 0.1 molar, was used as relaxation agent.

Isolation of Metabolites.—Penicillium herquei. CMI 112 950, was grown from a spore suspension in shaken culture for ten days in 250-ml conical flasks, each containing 100-ml of culture medium composed of 5% sucrose, 0.3% $NaNO_3$, 0.2% corn steep liquor, 0.1% KH_2PO_4, 0.1% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.005% $ZnSO_4 \cdot 7H_2O$, and 0.3% $CaCO_3$. The mycelium was filtered off and as much moisture as possible removed by pressing

on a Buchner funnel under suction. The mycelium was then transferred to a Soxhlet thimble and extracted with ether for 24 h. After drying, the ether was removed *in vacuo* to give a dark yellow solid which was acetylated by stirring overnight at room temperature with acetic anhydride in pyridine. The crude product mixture was purified by preparative thin-layer chromatography using silica GF_{254} plates (20 × 20 × 0.1 cm) eluted several times with acetone–light petroleum (15 : 100) as developing solvent. Deoxyherqueinone diacetate (3) was eluted with ethyl acetate from the least-polar band and recrystallised from methanol to give yellow needles, m.p. 174—176 °C (lit. m.p. 174—176 °C).

Herqueichrysin triacetate (8) was similarly eluted from the yellow band of intermediate polarity and crystallised from methanol to give yellow rods, m.p. 183—185 °C (lit.[6] m.p. 185 °C). Elution of the most-polar band gave *herqueichrysin monoacetate* (10) as yellow needles, m.p. 220—221 °C, from methanol (Found: C, 66.0; H, 5.6. $C_{22}H_{22}O_7$ requires C, 66.3; H, 5.6%); $\nu_{max.}$ 3 590, 3 400br, 1 765, 1 624, and 1 592 cm^{-1}; $\lambda_{max.}$ 235, 282, 325, and 432 nm (log ε 3.56, 3.56, 2.85, and 3.54). On further reaction with acetic anhydride in pyridine, herqueichrysin monoacetate was converted quantitatively into herqueichrysin triacetate.

Saponification of Herqueichrysin Triacetate.—Herqueichrysin triacetate (200 mg) was stirred for 12 h in M-ethanolic sodium hydroxide (5 ml). The solution was acidified, diluted with water, and extracted with ethyl acetate to give a yellow gum which crystallised from methanol to give herqueichrysin as yellow needles (120 mg), m.p. 174—175 °C (lit.,[6] m.p. 174 °C).

Incorporations of Sodium [1-^{14}C]*Acetate.*—Preliminary experiments indicated that phenalenone production in cultures of *P. herquei* commenced on the fourth day after inoculation and reached a maximum on the eighth day. Further experiments indicated that maximum incorporation of [1-^{14}C]acetate into the phenalenones occurred when the label was introduced in a series of additions on days 3, 4, 5, and 6 and the metabolites were isolated from 9-day old cultures. Thus when sodium [1-^{14}C]acetate (200 mg; 9.02×10^6 μCi $mmol^{-1}$) was added to 2 culture flasks in equal portions on days 3, 4, 5, and 6 and the mycelium harvested after 9 days, herqueichrysin triacetate (30 mg, 2.90×10^6 μCi $mmol^{-1}$) was obtained. This corresponds to a dilution factor of 3.1. On this basis it would be anticipated that equivalent feeding of [1-^{13}C]acetate (95%) would give herqueichrysin triacetate with an excess of 3.4% of ^{13}C-label over natural abundance at each labelled portion (assuming a total of 9 labelled portions in the molecule).

Incorporations of ^{13}C *Precursors.*—Sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate (200 mg) were added in aqueous solution to two shake cultures of *P. herquei* in equal portions on days 3, 4, 5, and 6 after inoculation; similarly with diethyl [2-^{13}C]malonate (250 mg) in ethanol. After 9 days growth the mycelium was harvested and deoxyherqueinone diacetate (*ca.* 30 mg) and herqueichrysin triacetate (*ca.* 70 mg) was isolated in each case.

The assistance of Mrs. M. Anderson and Mrs. J. Rothschild in the mycological work is gratefully acknowledged. Professor L. C. Vining kindly supplied a sample of authentic herqueichrysin.

[8/562 *Received*, 29*th March*, 1978]

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