THE BIOSYNTHESIS OF PHENALENONES

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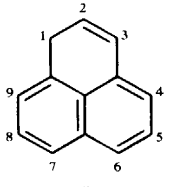
ABSTRACT

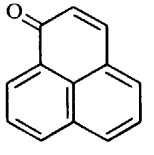
Over twenty phenalenones and related compounds have been isolated from fungi and from higher plants. The route of biosynthesis of the compounds isolated from fungi is however quite different from that of the phenalenones produced by green plants. The fungal phenalenones atrovenetin, herqueinone and norherqueinone are typical acetate-derived polyketides, the biosynthetic interrelationships of which have been clarified by the recent isolation of deoxyherqueinone from *Penicillium herquei*. In the monocot, *Haemodorum corymbosum*, on the other hand, the phenalenone nucleus is synthesised from two C_9 residues derived from shikimate, and a single acetate unit, possibly *via* a diarylheptanoid intermediate.

INTRODUCTION

The phenalene nucleus (I) was reported as a constituent of natural products in 1955 and 1956, by two groups concerned with structural investigations of apparently unrelated pigments produced by fungi imperfecti[1,2] and by an Australian monocotyledonous plant[3]. These initial studies described the occurrence of the phenalenone (II) nucleus in three fungal pigments, atrovenetin (IIIa), norherqueinone (IVa) and herqueinone (IVb) from *Penicillium herquei* and *P. atrovenetum*, the isolation and interrelationships of which had been previously established[4–6], and also the higher plant cellobioside haemocorin (Va) from the rhizome of *Haemodorum corymbosum* (Haemodoraceae).[3]

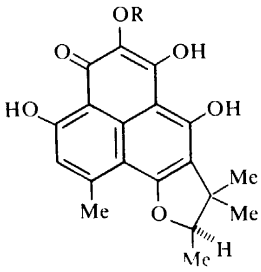


(I)

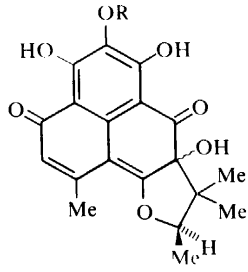


(II)

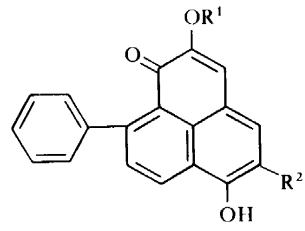
Comparatively few new natural products containing an intact phenalenone nucleus have been reported since these early publications, although a number of related naphthalene derivatives have been isolated from both fungi and higher plants, including several oxaphenalenes and one azaphenalene. The known products of this group are listed in *Table 1*, which



(IIIa) R = H
(IIIb) R = Me



(IVa) R = H
(IVb) R = Me



(Va) R^1 = cellobiose, R^2 = OMe
(Vb) R^1 = H, R^2 = OMe
(Vc) R^1 = R^2 = H
(Vd) R^1 = sugar, R^2 = OH

includes possible isolation artefacts such as the fungal naphthalic anhydride (VI)[7, 15] and isoherqueinone[10] (an epimer of herqueinone).

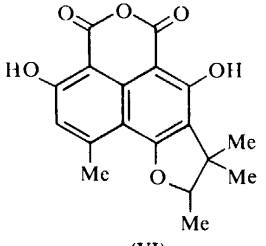
Precursor incorporation studies have shown that the structurally distinct fungal and plant phenalenones are also biosynthetically unrelated[12, 20, 21].

Table 1. Naturally occurring phenalenones

	Name	Source	Structure	Reference
1.	Atrovenetin	*Penicillium herquei* *P. atrovenetum*	IIIa	1, 2
2.	Deoxyherqueinone	*P. herquei*	IIIb	11
3.	Norherqueinone	*P. herquei*	IVa	2, 5
4.	Herqueinone	*P. herquei*	IVb	2, 4, 5, 6
5.	Isoherqueinone	*P. herquei*	Epimer of IVb	5, 10, 13
6.	*P. herquei* naphthalic anhydride	*P. herquei* *Fusicoccum putrefaciens*	VI	7, 15
7.	Duclauxin	*P. duclauxi*	VII	8
8.	Xenoclauxin	*P. duclauxi*	VIII	8
9.	Cryptoclauxin	*P. duclauxi*	IX	8
10.	2,7-Dihydroxy-5-methylnaphthaquinone	*Verticillium lamellicola*	X	9
11.	*V. lamellicola* chloro-naphthalic ester	*V. lamellicola*	XI	9
12.	Lamellicolic anhydride	*V. lamellicola*	XII	9
13.	*O*-Carbomethoxy-lamellicolic anhydride	*V. lamellicola*	XIII	9
14.	Resistomycin	*Streptomyces resistomycificus*	XXVI	16
15.	Haemocorin	*Haemodorum corymbosum*	Va	3, 14
16.	Haemocorin aglycone	*H. corymbosum*	Vb	12
17.	Lachnanthocarpone	*Lachnanthes tinctoria*	Vc	17
18.	Lachnanthoside	*L. tinctoria*	Vd	17, 18
19.	*N*-(2-hydroxyethyl) lachnanthopyridone	*L. tinctoria*	XVa	18
20.	Lachnanthopyrone	*L. tinctoria*	XVb	16
21.	*L. tinctoria* naphthalide	*L. tinctoria*	XVI	16
22.	*L. tinctoria* naphthalic anhydride	*L. tinctoria*	XIV	19
23.	Lachnanthofluorone	*L. tinctoria*	XXV	17

NATURALLY OCCURRING PHENALENONES AND RELATED PRODUCTS

The present review is primarily concerned with the biosynthesis of phenalenones, and since previous articles[14, 22, 23] have detailed the chemistry of phenalenes and the structural elucidation of naturally-occurring phenalenones, only chemical aspects of biosynthetic relevance will be considered.



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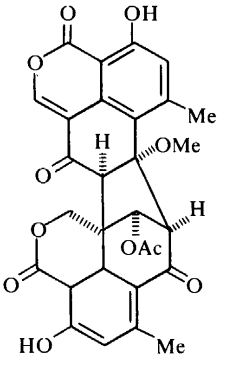

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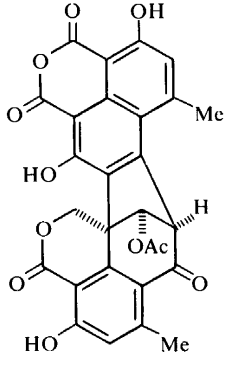

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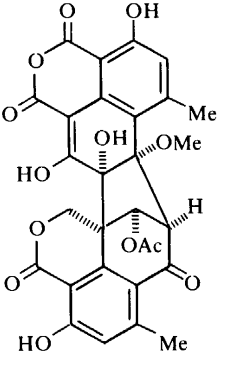

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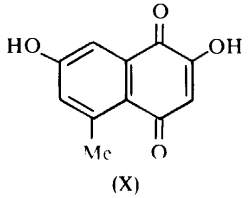
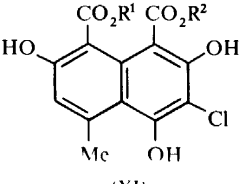
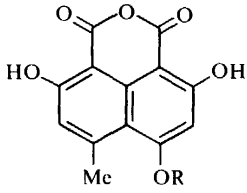


(X)

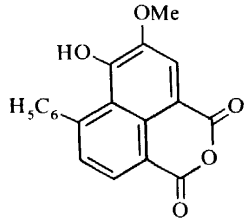


(XI)

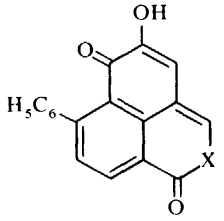
R^1 = Me, R^2 = H
or R^1 = H, R^2 = Me



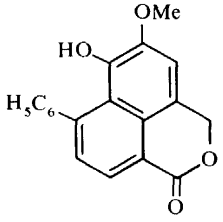
(XII) R = H
(XIII) R = COOMe



(XIV)



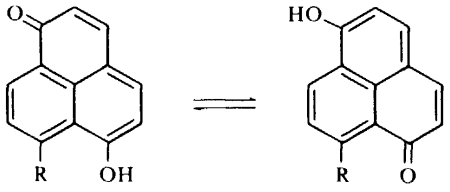
(XVa) X = N–CH_2–CH_2OH
(XVb) X = O



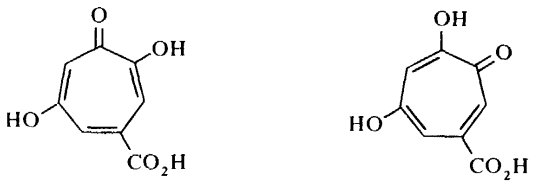
(XVI)

An important consequence of the presence of a carbonyl group in the phenalenone nucleus, is that it allows the carbon skeleton which contains an odd number of atoms (C_{13}), to assume a fully conjugated structure. When suitably hydroxylated, this nucleus can exist in different tautomeric forms, for example (XVIIa) and (XVIIb). This behaviour is also exhibited by the tropolone nucleus (C_7), which is present in another small group of natural products, e.g. stipitatic acid (XVIIIa ⇌ XVIIIb) from *Penicillium stipitatum*.

Whereas atrovenetin and deoxyherqueinone can be represented as fully conjugated structures, the conjugation is interrupted in herqueinone and norherqueinone by the presence of a tertiary hydroxyl group. This substituent,



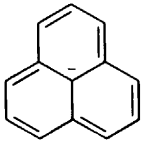
(XVIIa) (XVIIb)



(XVIIIa) (XVIIIb)

which appears to be associated with the susceptibility of the C_5 moiety to acid cleavage in herqueinone and norherqueinone, is removed on reduction with zinc in acetic acid at room temperature, with the formation of deoxyherqueinone or atrovenetin respectively.

The phenalene nucleus is unusual in that it can give rise to a relatively stable anion (XIX), cation (XX) or radical (XXI) by the respective loss of a proton, a hydride ion, or a radical. Such considerations led to the successful prediction that suitable hydrocarbon derivatives, e.g. the indeno-[2,1-a] phenalene (XXII)[24, 25] would possess aromatic character. Phenalenes undergo facile oxidation to phenalenones even on exposure to air. The phenalenone nucleus, which incidentally exhibits considerable basicity ($pK_b = 0.4$)[26] is comparatively stable, although oxidative cleavage leading to naphthalic anhydrides takes place under fairly mild conditions. Thus atrovenetin (IIIa) and deoxyherqueinone (IIIb) are readily converted to the naphthalic anhydride (VI), either with alkaline hydrogen peroxide[2] or alternatively by photochemical oxidation[27]. In view of this latter report[27], and the observation by Kriegler and Thomas (unpublished results) that in crude fungal extracts containing atrovenetin and deoxyherqueinone, the concentration of the anhydride is initially very low and increases with time of storage, it is possible that the reported natural anhydride[7, 15] may arise, at least in part, as an artefact of the isolation procedure.



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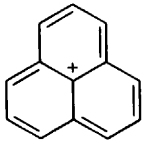
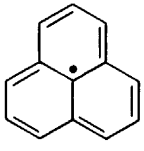
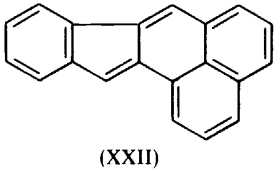


(XX)



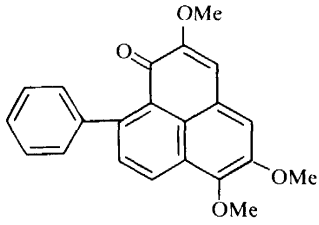
(XXI)



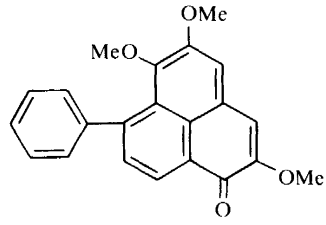
(XXII)

The plant phenalenones, in contrast to their fungal counterparts, all possess a phenyl substituent. The orientation of the hydroxyl substituents allows the existence of two tautomeric forms of haemocorin aglycone, the corresponding dimethyl ethers of which (XXIIIa) and (XXIIIb) have been prepared[3]. Subsequent structure assignments of haemocorin-related products were facilitated by the mass spectral observation that a phenalenone with a phenyl substituent *peri* to the carbonyl group as in (Va) exhibits a predominant $(M - 1)^+$ ion,[20] the provisional structure of which (XXIV) was suggested by Shannon[28]. Thus it was observed that the methyl ethers (XXIIIa) and (XXIIIb) yielded spectra characterized by major $(M - 1)^+$ and M^+ peaks respectively[20, 29]. A product related to structure (XXIV),

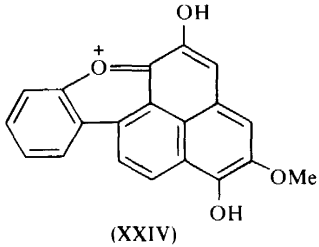
namely lachnanthofluorone (XXV), has been prepared by Weiss and Edwards[17] by photochemical oxidation of the aglycone of its co-metabolite, the *Lachnanthes tinctoria* pigment lachnanthoside (Vd). Mass spectrometric studies by Waight[29] have demonstrated that a prominent $(M - 1)^+$ ion is a characteristic of other compounds with *peri*-orientated phenyl and carbonyl groups, such as 2-phenylanthraquinone.



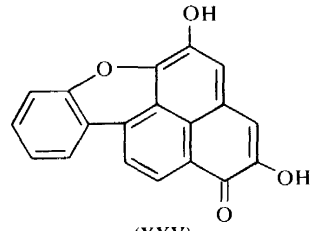
(XXIIIa)



(XXIIIb)



(XXIV)



(XXV)

The phenalenone pigments have been the subject of synthetic studies. A partial synthesis of atrovenetin was described by Bycroft and Eglington[30] and recently a complete synthesis of the racemate was reported by Frost and Morrison[31]. Morrison and co-workers have demonstrated the absolute *R*-configuration of atrovenetin (IIIa) and have also effected a synthesis of the aglycone of the plant phenalenone haemocorin[32] (Vb). The configurational relationship of herqueinone and its epimer isoherqueinone, both of which contain two chiral centres, has been investigated by the groups of Morrison[13] and Cason[10], who however arrived at different conclusions. The position of the methoxyl substituent has also been investigated by these two groups,[10, 33] both of which favour the orientation shown in structure (IVb).

An additional microbial product containing the phenalenone nucleus is resistomycin (XXVI)[16] although the structural relationship can only be regarded as a superficial one, since the phenalenone moiety is part of a pentacyclic structure based on a C_{19} hydrocarbon.

A new group of what appear to be phenalenone-derived metabolites of *Verticillium lamellicola*, structures (X) to (XIII), has recently been discovered by McCorkindale and co-workers[9]. In addition to novel features such as the chloro substituent (XI) and an *O*-carbomethoxy group (XIII), these compounds are notably distinct from the *P. herquei* products in that they are devoid of the C_5-unit of the latter series, as is also the case with the dimeric *P. duclauxi* pigments (VII), (VIII) and (IX).[8]

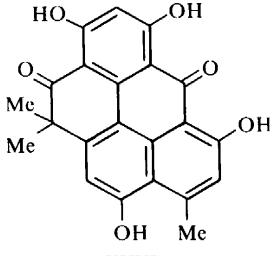
BIOSYNTHESIS OF PHENALENONES

The speculative proposal[20] that plant and fungal phenalenones are biosynthesized from different primary precursors has now been substantiated[12]. Consequently the biosynthetic studies of these two groups will be discussed separately.

Fungal phenalenones

Primary precursors

Structure analysis considerations led Barton *et al.*[2] to suggest that atrovenetin, initially formulated as (XXVII), was an example of the then recently recognized group of acetate-derived phenolic metabolites (now classified as polyketides or acetogenins). The C_5-substituent was envisaged as an isoprenoid unit of mevalonate origin, a biosynthetic theory more readily satisfied by the alternative structure (IIIa)[20], which was subsequently confirmed by an x-ray crystallographic analysis of the ferrichloride of the trimethyl ether of atrovenetin[34].



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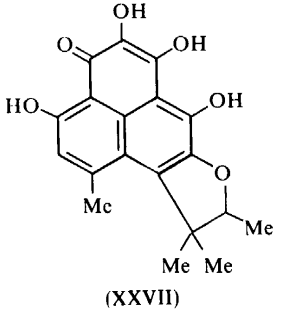
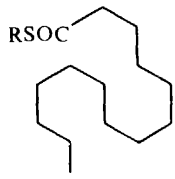


(XXVII)

The carbocyclic nucleus of the fungal phenalenones could in addition be considered to arise via a C_6–C_3–C_6 chalcone-type intermediate.[12] However, its wholly acetate origin as predicted by Barton *et al.*[2], was subsequently established through a study of the biosynthesis of norherqueinone (IVa), which also confirmed the derivation of the C_5 side chain from mevalonate[12].

The nature of the acyclic intermediates leading from acetate and malonate to the phenalenone ring system, in common with the majority of phenolic polyketides, is unknown. If it is assumed that a linear C_{14}-heptaketide is involved, then there are three alternative possible modes of folding which can in theory lead to a methylphenalenone nucleus, namely (XXVIII), (XXIX) and (XXX). No naturally occurring mono- or bi-cyclic heptaketide products have been isolated from phenalenone-producing fungi which resemble potential intermediates.

The dimeric *Penicillium duclauxi* metabolites, namely duclauxin (VII), xenoclauxin (VIII) and cryptoclauxin (IX), bear an obvious structural relationship to the known methylphenalenones and have also been shown to be acetate-derived[8]. If each monomeric C_{13}-unit originates from a linear C_{14}-heptaketide, then its formation most likely involves the oxidative ring fission of a tricyclic intermediate of the phenalene type. At present, there is no indication as to the stage at which coupling of the monomer units takes



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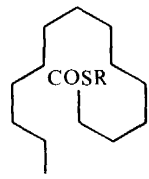
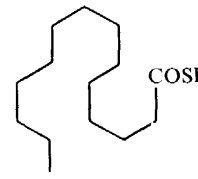


(XXIX)



(XXX)

place. This could involve either coupling of two identical methylphenalenones followed by independent oxidative ring cleavages, or alternatively the direct coupling of two different oxaphenalenones.

The cyclizations of the acetate–polymalonate precursors of phenolic polyketides, are generally considered to involve a combination of Claisen and aldol condensations, as indicated in *Figure 1* (path a) for the hypothetical conversion of tetraacetyl coenzyme A (XXXI) to orsellinic acid (XXXII). A direct extension of this process could account for the formation of all the carbocyclic polyketides, including the fungal phenalenones.

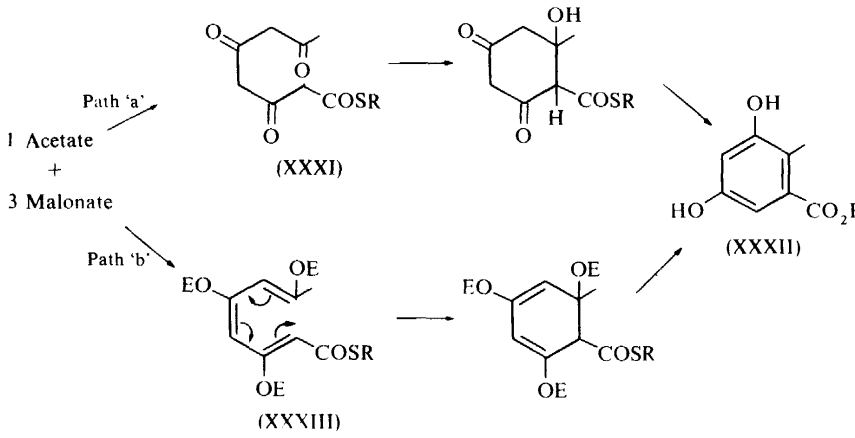


Figure 1. Cyclization of polyketides.

An alternative mechanism, *Figure 1* (path b), could however generate polyketide phenols without the mediation of ketonic intermediates. This involves the formation of an enzyme-stabilized poly-β-enolate (XXXIII) with the appropriate geometry required to allow it to undergo an electrocyclic rearrangement to an intermediate from which orsellinic acid is formed by a simple elimination process. In the case of the phenalenone nucleus, the precyclization intermediate (XXXIV) could undergo conversion to (XXXV) in a single concerted cyclization step (*Figure 2*). Subsequent elimination of water (or the bound enzymes) would then yield the phenalenone (XXXVI). The actual mode of cyclization of any poly-β-enolate, would be predetermined by the sequence of *cis* and *trans* double bonds, the formation of which

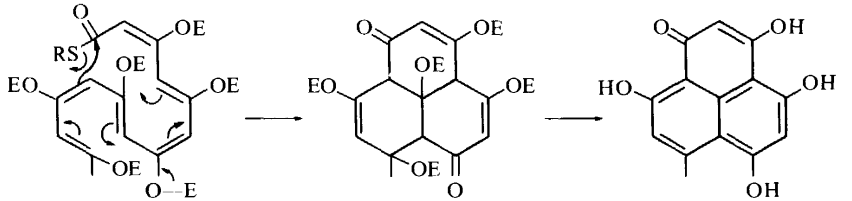


Figure 2. Cyclization of the phenalenone precursor.

may be dependent upon the stereospecificity of the elimination of the thioester group from the polyketide intermediates, as shown in *Figure 3*.

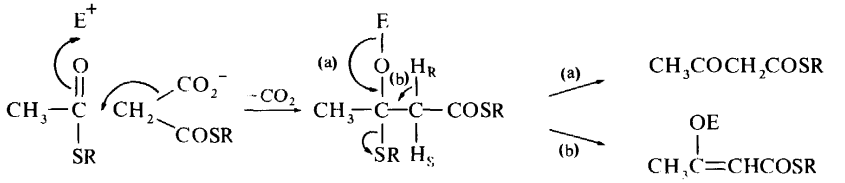


Figure 3. Stereospecificity of polyenolate formation.

Advanced intermediates

The production of phenalenones by fungi was discovered as a consequence of structural studies of atrovenetin (IIIa), norherqueinone (IVa) and herqueinone (IVb). Isoherqueinone was initially observed as a product of the base-catalyzed epimerization of herqueinone;[5] it was subsequently detected in crude extracts of *P. herquei*[10], although the possibility that it is an artefact of the isolation process has not been excluded.

The only new fungal phenalenone to be isolated since this early work is deoxyherqueinone (IIIb), prior to the detection of which, it was reasonable to assume that herqueinone was probably derived from atrovenetin via norherqueinone by way of sequential oxidation and methylation, *Figure 4* (path a). Deoxyherqueinone was only recognized as a normal metabolite of *P. herquei*, following a search for new phenalenones and their intermediates, based on an examination of the products formed on incubating with ^{14}C-labelled acetate, formate and mevalonate, in conjunction with radio-autographic procedures[11]. The direct isolation of this fungal constituent by preparative thin layer chromatography, was unsuccessful due to the low yield and comparative instability of the product. However, its isolation by counter-current distribution techniques proved to be more satisfactory.

With the characterization of deoxyherqueinone, an alternative possible pathway for the conversion of atrovenetin to herqueinone, via sequential methylation and oxidation, *Figure 4* (path b), became apparent. Examination of the relative efficiencies of interconversion of these four phenalenones by *P. herquei*,[35] substantiated the operation of this latter pathway, to the apparent exclusion of the alternative path a. Thus it was observed that norherqueinone, although derived from atrovenetin, was not methylated to

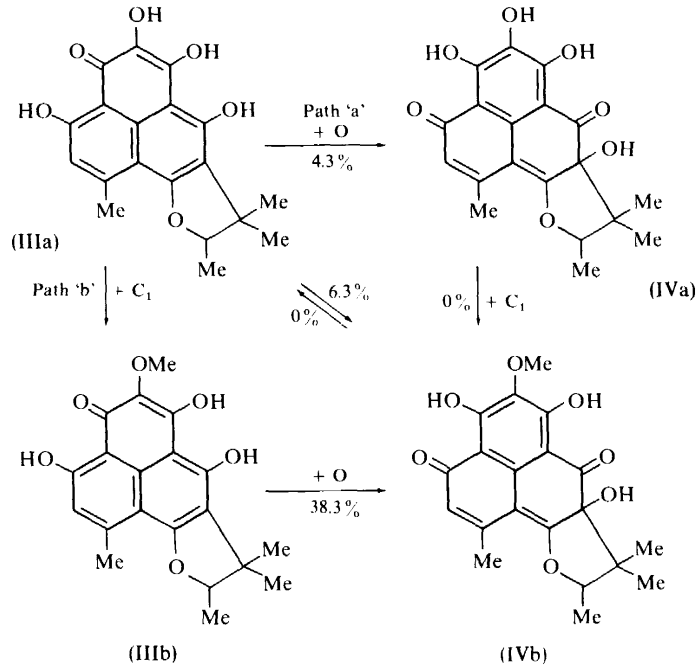


Figure 4. Interrelation of fungal phenalenones.

form herqueinone, whereas deoxyherqueinone was incorporated into herqueinone with an efficiency of approximately 38 per cent.

Oxidative metabolism of phenalenones can apparently take place in *P. duclauxi* leading to the formation of the dimeric oxaphenalenes, such as duclauxin (VII); it may also occur in *P. herquei*, *Fusicoccum putrefaciens* and *Verticillium lamellicola* which produce naphthalic acid derivatives, although as previously mentioned, a corresponding non-enzymatic oxidative ring cleavage of phenalenones can take place under photooxidative conditions.

Higher plant phenalenones

Only the primary precursors of the higher plant products have thus far received experimental examination. Following the demonstration of the polyketide nature of the fungal phenalenones,[12] the possibility of a similar origin of the plant phenalenone haemocorin aglycone (Vb) was proposed,[36] *Figure 5* (path a). However, structural analysis considerations, based on the presence of a phenyl group and two sets of vicinal oxygen substituents, had led to the suggested derivation of the C_{19}-phenylphenalenone nucleus from two C_6–C_3 units and one acetate unit *Figure 5* (path b)[12] via a bisaryl-heptanoid intermediate (XXXVII), closely related to curcumin (XXXVIII), a constituent of the rhizome of the monocotyledon *Curcuma longa*.

Evidence supporting the formation of haemocorin aglycone by this latter pathway has been obtained through ^{14}C-labelled precursor feeding studies, which demonstrated the specific incorporation of tyrosine [2-^{14}C] with

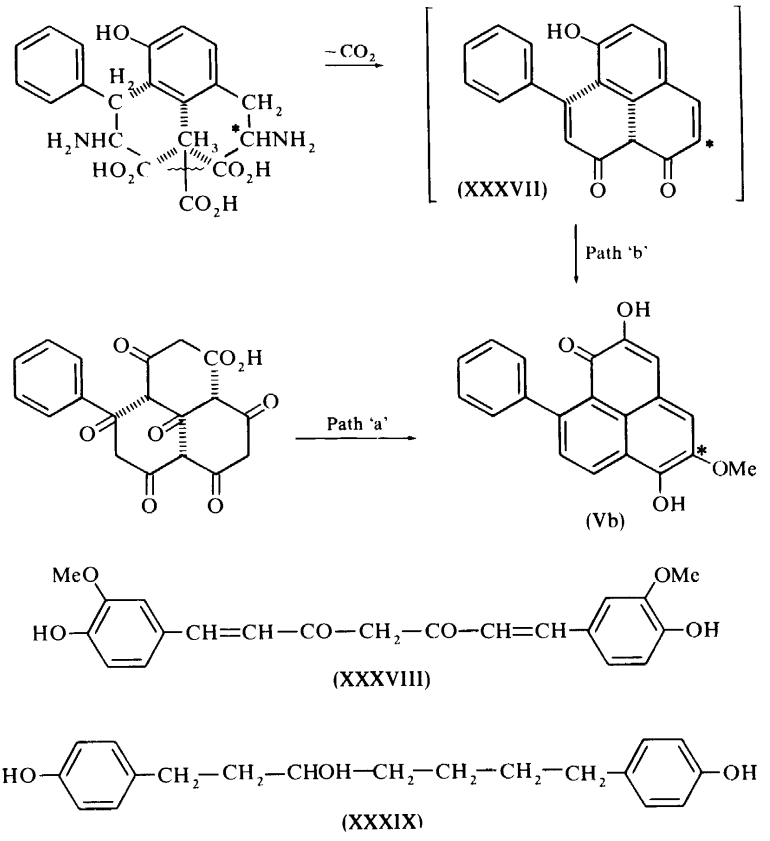


Figure 5. Biosynthesis of higher plant phenalenones.

approximately 0.50 per cent efficiency.[20] All the resulting ^{14}C-activity was located at the predicted position (C-6) by means of a specific degradation sequence based on the original chemical studies of Cooke and his collaborators[3,14]. In addition, it was observed that both phenylalanine [1- and 2-^{14}C] were incorporated with comparable efficiencies (0.29 and 0.30 per cent respectively), as to a lesser extent was acetate [2-^{14}C], consistent with the bisarylheptanoid pathway. The absence of suitable small scale chemical degradation procedures, precluded an examination of the specificity of incorporation of these latter precursors. A lower yet significant uptake of acetate [1-^{14}C] was also observed, which may be the result of its secondary incorporation into C_6–C_3 units, as has been reported in biosynthetic studies of various alkaloids known to originate from the shikimic acid-derived aromatic amino acids.

These data are entirely in accord with the precursor incorporation results obtained by Edwards, Schmitt and Weiss[21] in an extensive examination of the incorporation of ^{14}C-labelled precursors into the phenylphenalenone nucleus of lachnanthoside (Vd). This is a glycosidic constituent of the roots

of *Lachnanthes tinctoria* Ell., which is the only haemodoraceous plant found in the temperate zone of the Northern Hemisphere. Although the positions of the labelled atoms were not located, the relatively efficient incorporation of the various C_6–C_3 precursors and of acetate [2-^{14}C], leaves little doubt that these function as primary precursors. Again, some acetate [1-^{14}C] uptake was observed, (0.24 per cent incorporation efficiency relative to 0.62 per cent for acetate [2-^{14}C]). However, the possibility of a specific involvement of the acetate carboxyl carbon in place of either the carboxyl of tyrosine or phenylalanine, is inconsistent both with the efficient utilization of tyrosine [1-^{14}C] (0.47 per cent), which approximated to that of tyrosine [3-^{14}C] (0.58 per cent) and also the particularly high incorporation (3.80 per cent) of phenylalanine [1-^{14}C] which corresponded closely to that of the [3-^{14}C]-labelled amino acid (3.48 per cent).

The nature of the immediate precursors of the plant phenalenones remains to be determined, although the proposed mediation of diarylheptanoids of the curcumin type remains an attractive hypothetical possibility. The Zingerberaceae, which includes the genus *Curcuma*, and also the Haemodoraceae are both monocotyledonous families. However, the significance of this possible chemotaxonomic relationship is apparently reduced by the known occurrence of diarylheptanoids in dicotyledons, such as centrolobol (XXXIX) from *Centrolobium spp.* (Leguminosae). In this latter regard, it may be chemotaxonomically significant, that the diarylheptanoids from dicotyledonous genera appear to be more highly reduced than their monocotyledonous counterparts (the known examples of diarylheptanoids have been listed[21]), so that these latter products are closer to the higher oxidation level required for phenalenone formation.

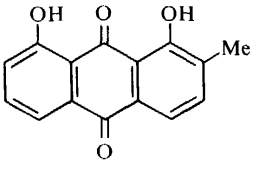
The first study of the biosynthesis of diarylheptanoids has recently been reported by Roughley and Whiting,[37] on the basis of which they have suggested that in curcumin (XXXVIII), one benzene moiety is derived from the normal C_6–C_3 precursors, but that the other may be of polyketide origin. In view of the structural symmetry of this product, such an unsymmetrical biosynthetic origin would be unexpected, and until the typical polyketide distribution of labelling from acetate or malonate [1- or 2-^{14}C]-derived curcumin is established, the possibility of indirect secondary labelling from these precursors cannot be excluded, as has been proposed to account for the significant incorporation of acetate [1-^{14}C] into haemocorin aglycone[20] and lachnanthoside[18].

No direct evidence is available concerning the further metabolism of the phenylphenalenones. However, the discovery of oxa and azaphenalene derivatives (XVa), (XVb) and (XVI) in *Lachnanthes tinctoria*[18], together with the phenylnaphthalic anhydride (XIV)[19] (*Table 1*), is indicative of an enzymatic phenalenone oxidative ring cleavage system in this plant, although haemocorin has been shown to undergo photolytic degradation[27], in an analogous manner to the fungal phenalenones.

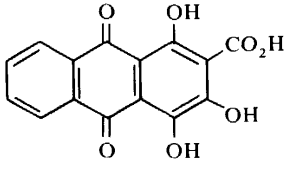
CONCLUSIONS

The discovery that the phenalene tricyclic ring system can be biosynthesized by two unrelated primary pathways finds an analogy in the observation

that some polyhydroxyanthraquinones are typical polyketides, such as emodin and chrysophanol (XL)[38] whereas others, for example alizarin and pseudopurpurin (XLI) have been shown to be derived from shikimate[39] and mevalonate.[40] However, at present no conclusive example appears to have



(XL)



(XLI)

been reported of any individual polycarbocyclic phenol or quinone arising from different primary precursors, and it may be that this apparent specificity of origin is a general characteristic of the majority of natural products, with only infrequent exceptions such as the amino acid lysine, for which different biosynthetic pathways have evolved in the bacteria and the fungi[41].

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