The Biosynthesis of Pachybasin (1-hydroxy-3-methylanthraquinone), a Metabolite of *Phoma foveata*

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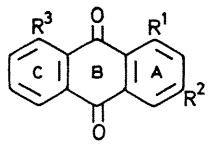
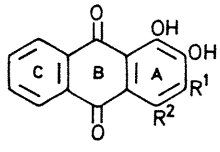
Summary Incorporation experiments using [2-^{14}C]- and [1-^{14}C]-acetate and [2-^{14}C]-malonate have established that the biosynthesis of the fungal anthraquinone, pachybasin (**4**), involves an acetate–malonate pathway.

THERE are two distinct routes for the biosynthesis of the carbon skeleton of anthraquinones of higher plants. One is represented by the formation of chrysophanol (**1**) in *Rumex alpinus* and *Rhamnus frangula* through an acetate–malonate pathway[1] and the other by the derivation of alizarin (**2**) and purpurin carboxylic acid (**3**) from shikimic acid through a naphthaquinone precursor (for rings B and C) and an isoprenoid unit (for ring A).[2,3] For fungal anthraquinones, cases investigated, indicate that the biosynthesis occurs by the acetate–malonate pathway.[4] It was of interest, therefore, to investigate the biosynthesis of pachybasin (**4**),[5–7] a fungal metabolite which has ring C of the nucleus unsubstituted like the plant anthraquinones (**2**) and (**3**) derived from shikimic acid.





(1) $R^1 = R^3 = OH$, $R^2 = Me$ (2) $R^1 = R^2 = H$)
(4) $R^1 = OH$, $R^2 = Me$, $R^3 = H$ (3) $R^1 = CO_2H$, $R^2 = OH$)
(5) $R^1 = OAc$, $R^2 = Me$, $R^3 = H$
(6) $R^1 = OAc$, $R^2 = CO_2H$, $R^3 = H$
(7) $R^1 = OH$, $R^2 = CO_2H$, $R^3 = H$
(8) $R^1 = OH$, $R^2 = R^3 = H$

In this study, sodium [2-^{14}C]-acetate, sodium [1-^{14}C]-acetate and diethyl [2-^{14}C]-malonate were incorporated, separately, during 48 h into 4-day cultures of *Phoma foveata*. Samples of [^{14}C]-pachybasin obtained in each of these three experiments were oxidized to phthalic acid by potassium permanganate in alkaline solution and, separately, converted through a known degradation sequence (**4** → **5** → **6** → **7** → **8**)[5] into 1-hydroxyanthraquinone (**8**). Measurements of the radioactivities of starting material and products made it possible to deduce that the [^{14}C]-acetate and [^{14}C]-malonate had been incorporated into rings A, B, and C of pachybasin (Table).

TABLE

Labelled substrate	*Assays of radioactivity* [2-^{14}C]-acetate		[1-^{14}C]-acetate		[2-^{14}C]-malonate	
Degree of incorporation of label	0·54%		0·26%		0·25%	
Pachybasin, [^{14}C]-activity	1·463[a]	100%	0·495[a]	100%	0·96[a]	100%
1-OH-Anthraquinone	1·15[a]	79%	0·488[a]	98·7%	0·80	83%
Me group at C-3		21%		1·3%		17%
Average activity at 7 active sites		11·2%		12·8%[b]		11·9%
Phthalic anhydride	0·657[a]	45%	0·257[a]	51·8%	0·460[a]	48%
Average activity at 4 active sites		11·2%		11·7%		12%

[a] Measured in d.p.m. × 10^6 per μ mol. [b] Assuming 1·3% per inactive site.

The difference in radioactivity in pachybasin, and the 1-hydroxyanthraquinone derived from it, provided a basis for calculating the "starter group effect" of the methyl group. As expected for an acetate–malonate pathway, when [2-^{14}C]-acetate was incorporated, this methyl group carried more [^{14}C]-label than the average labelled position but there was no such effect for [1-^{14}C]-acetate incorporation. Having taken this effect into account, where necessary, there was good agreement between the average activities calculated for four labelled centres in the phthalic anhydride and seven in the 1-hydroxyanthraquinone derived from [^{14}C]-pachybasin. This indicated that pachybasin was formed in *Phoma foveata* from acetate and malonate units.

The incorporation experiments using [2-^{14}C]-diethyl malonate gave anomolous results. The methyl group at C-3 had more ^{14}C-label (17%) than the average (12%) for labelled positions in the nucleus whereas, if it is assumed that the biosynthesis was initiated by an acetate unit, this methyl group would be expected to carry less than the average label. The result may be explained by ready decarboxylation of malonate by the micro-organism, as suggested for another case.[8] However, such examples indicate that the use of [2-^{14}C]-malonate to identify "starter groups" may produce ambiguous results.

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