

BIOSYNTHESIS OF RAVENELIN FROM [1-¹³C]-AND [1,2-¹³C]-ACETATE

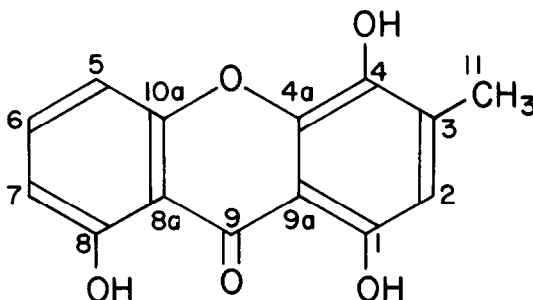
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Ravenelin (1) is a fungal metabolite from some *Helminthosporium* species.^{1,2}



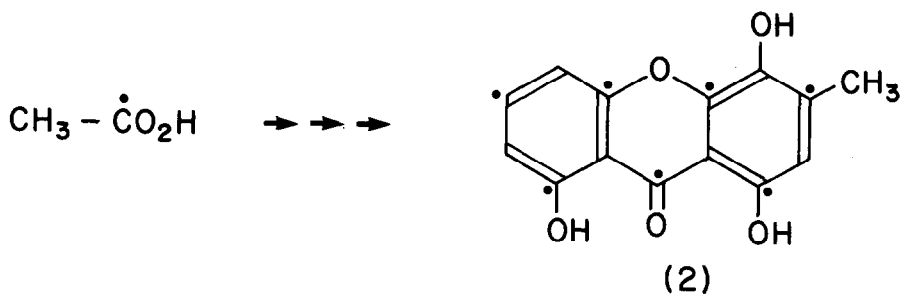
(1)

An early suggestion³ was that compounds with a benzophenone skeleton are biosynthesised by two types of route: wholly acetate-polyketide, or shikimate-polyketide (C_6C_1 plus 3- C_2 or C_6C_3 plus 2- C_2). The former route is probable in fungi and the latter in higher plants,⁴ a conclusion supported by biosynthetic studies.^{5,6}

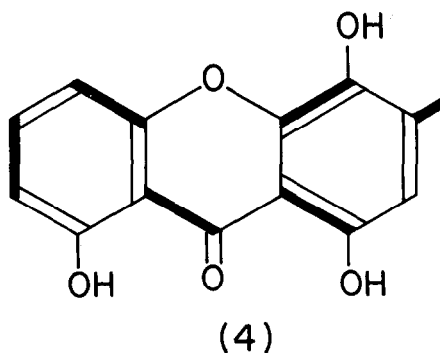
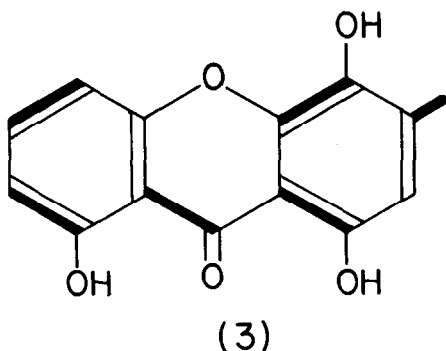
A preliminary investigation of ravenelin using [¹⁴C]-acetate supported a complete acetate-polyketide origin but was not definitive of detail.⁷

A culture of *H. ravenelii* has now been fed with sodium [1-¹³C]-acetate (57%) or with sodium [1,2-¹³C]-acetate (91.6% and 93.1% respectively) and the resulting ravenelin, which was enriched in ¹³C- abundance about twofold (mass spectrometry), was examined by ¹³C-nmr spectroscopy.

The results from feeding experiments with [1-¹³C]-acetate are in complete agreement with the origin shown in (2) (see Table 1)



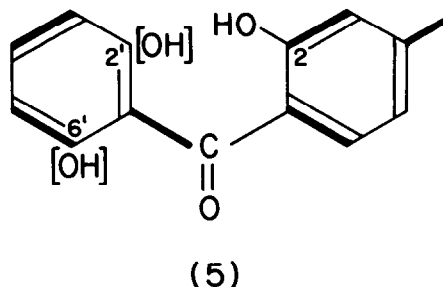
The proton-noise-decoupled ^{13}C -n.m.r. spectrum of ravenelin enriched with $[1,2-^{13}\text{C}]$ -acetate showed the presence of intense satellite resonances due to ^{13}C - ^{13}C spin-spin coupling between C_{11} - C_3 , C_4 - C_{4a} , C_9 - C_{8a} and C_1 - C_{9a} indicating their origin from four intact acetate units. C_5 also shows intense satellites indicating origin from an intact acetate unit. However these satellites are very broad, due to coupling of C_5 to both C_6 and C_{10a} to the same extent. Similarly C_7 is coupled to both C_6 and C_8 . These observations indicate that C_{10a} , C_5 , C_6 , C_7 and C_8 are derived from two intact acetate units distributed in equal amounts as shown in (3) and (4). In agreement with this,



the relative intensities of the C_8 and C_{10a} satellites are only half those of the remaining nuclei derived from intact acetate units (Table 2). C_2 shows no intense satellites indicating its origin from a cleaved acetate unit.

The high value for the satellite intensities of C_{11} in Table 2 arises because the satellite signals are not further split by ^{13}C - ^{13}C spin-spin coupling with adjacent intact acetate units. Such coupling is observed for other signals because of a relatively high level of acetate incorporation. Satellites of the C_2 signal arise from spin-spin coupling of the C_2 nucleus with adjacent acetate units (C_{11} - C_3 and C_1 - C_{9a}) so a low value is anticipated.

The above results indicate that an oxygenated benzophenone derivative (5),



of polyketide origin, is an intermediate in the biogenesis of ravenelin. The observed labelling distribution results from the equal probability of cyclization between positions 2 and 2', or 2 and 6' to give the xanthone skeleton.

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References

1. H. Raistrick, R. Robinson, and D.E. White, *J. Biochem.*, **30**, 1303 (1936).
2. V. Kahlwalia and T.R. Seshadri, *Proc. Indian Acad. Sci.*, **44A**, 1 (1965); *Chem. Abstracts*, **51**, 5059 (1957).
3. A.J. Birch and F.W. Donovan, *Aust. J. Chem.*, **6**, 360 (1953).
4. O.R. Gottlieb, *Biochem. Systematics*, 1973, **7**, 111.
5. A.J. Birch, R.A. Massey-Westrop, R.W. Rickards and H. Smith, *J. Chem. Soc.*, 1958, 3606; W.J. McMaster, A.I. Scott and S. Trippett, *J. Chem. Soc.*, 1960, 4628; J.S.E. Holker and S.A. Kagal, *Chem. Comm.*, 1968, 1574; K.G.R. Pachler, P.S. Steyn, R. Vleggaar and P.L. Wessels, *J.C.S. Chem. Comm.*, 1975, 355, and references cited therein; A.G. McInnes, D.G. Smith, J.A. Walter, L.C. Vining and J.L.C. Wright, *J.C.S. Chem. Comm.*, 1975, 66; P. Gupta and J.R. Lewis, *J. Chem. Soc. (C)*, 1971, 629.
6. J.S.E. Holker, R.D. Lapper and T.J. Simpson, *J.C.S. Perkin I*, 1974, 2135.
7. G.E. Blance, Ph.D. Thesis, Manchester University, 1960; S.F. Hussain, Ph.D. Thesis, Manchester University, 1964.

TABLE 1

^{13}C -Chemical shifts of ravenelin, excess ^{13}C -abundance at individual positions in $[1-^{13}\text{C}]$ -acetate enriched ravenelin, and coupling constants ($^1\text{J}_{^{13}\text{C}-^{13}\text{C}}$ /Hz) of $[1,2-^{13}\text{C}]$ -acetate enriched ravenelin.

Carbon	Signal*	E#	$^1\text{J}_{^{13}\text{C}-^{13}\text{C}}$
1	151.7	9.6	64.1
2	111.1d	0.7	56.8
3	136.9	14.1	42.1
4	135.0	1.9	76.9
4a	143.7	6.9	78.7
5	107.4d	1.1	-62 ⁺
6	137.7d	4.4	56.8
7	110.3d	0.6	-62 ⁺
8	160.4	10.7	69.6
8a	107.2	1.0	53.8
9	185.2	9.8	54.9
9a	105.9	1.2	64.1
10a	155.7	10.6	67.7
11	17.0q	0.4	42.1

* Determined on 0.09M solutions of unenriched ravenelin at ambient probe temperature (37^o); in p.p.m. downfield from Me_4Si ; measured from internal d_6 -DMSO and corrected by using the expression $\delta_{\text{TMS}} = \delta_{\text{DMSO}} + 39.6$. Multiplicities are indicated from the proton off-resonance decoupled spectrum.

For method of calculation see reference 6.

+ Broad unresolved signals.

TABLE 2

Ratio of satellite intensities to natural-abundance peak intensities in [1,2-¹³C]-acetate enriched ravenelin.

Carbon	Ratio
1	1.2
2	0.6
3	1.5
4	1.6
4a	1.4
5	1.4
6	1.5
7	1.5
8	0.7
8a	1.4
9	1.4
9a	1.5
10a	0.8
11	2.5