Fatty Acid Cyclization in the Biosynthesis of Brefeldin-A: a New Route to some Fungal Metabolites

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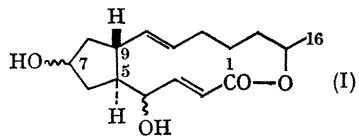
Summary Specific incorporation of ^{14}C from [9-^{14}C]palmitate into the antibiotic brefeldin-A is observed, and explained in terms of biosynthesis *via* an oxygenative 5,9-cyclization of a 1,15-lactonized C_{16}-polyene acid.

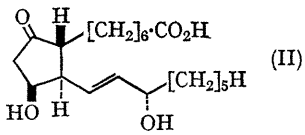
WE report biosynthetic studies on the polyketide antibiotic brefeldin-A (I). For the aromatic polyketides, biosynthesis is thought to involve the assembly of enzyme-bound polyketo-acyl chains which can undergo some limited modifications before being cyclized and released from the enzyme system. The repeated failure of experiments designed to show intact conversion of appropriate fatty acids into such polyketides is generally regarded as significant. However, for the more highly reduced polyketides (of which the natural fatty acids are the ultimate example) the possibility that they may be further metabolites of common fatty acids still merits consideration. An increasing range of structural modifications can now be shown to arise by such means, for example in the natural acetylenes or, more immediately comparable to (I), the prostaglandins, *e.g.* (II).

The overall derivation of (I) from acetate has been demonstrated by Coombe *et al.*,[1] and was confirmed by us in preliminary trials. We used the reductive ozonolysis procedure developed by Sigg[2] to obtain the degradation products listed in the Table; the carbon atoms of (I) are here numbered as for a fatty acid, *i.e.* serially along the unbranched chain from the carbonyl carbon. We used *Penicillium cyaneum* for the production of (I), in submerged culture as described by Fuska and Kuhr.[3] At 120 hr. from inoculation, [9-^{14}C]palmitate[4] (300 μc) was added, and the brefeldin-A was isolated 10 hr. (incorporation 12%) later; in another experiment (noted below) the incubation with [^{14}C]palmitate lasted 40 hr.

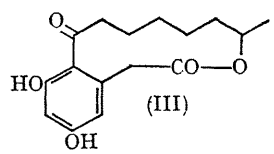
The results reveal two pathways for the transfer of ^{14}C from [9-^{14}C]palmitate to (I). The first is by β-oxidation followed by synthesis from the [1-^{14}C]acetate thus formed,



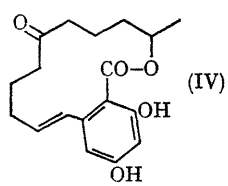
(I)



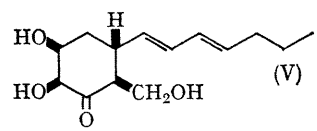
(II)



(III)



(IV)



(V)

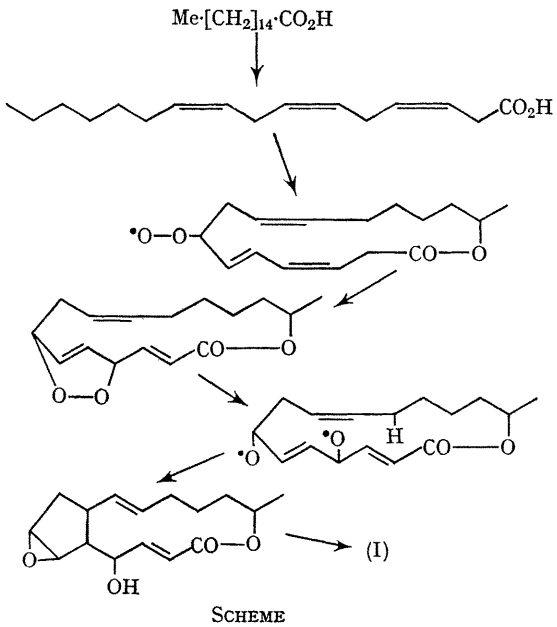
giving substantially uniform labelling of the odd-numbered atoms of (I), as in the previous work in which [1-^{14}C]acetate was used directly. The second pathway, and in this experiment the more important, leads to specific labelling

TABLE

Degradation of brefeldin-A labelled from [9-^{14}C]palmitate

	C atom nos.[a]	R.m.a. × 10^{-4}	Ratio[b]	Excess
Brefeldin-A	1—16	15·30	10·85	2·85
Hexane-1,5-diol bis-*p*-nitrobenzoate	16—11	4·23	[3·0]	
Ethane-1,2-diol bis-*p*-nitrobenzoate	1—2	1·3[c]	1·1	
3-(1,2-Dihydroxrethyl)-4-hydroxymethylcyclopentanol tetra-*p*-nitrobenzoate	3—10	9·80	6·95	2·95
3,4-Dimethoxycarbonylcyclopentanone-2,4-dinitrophenylhydrazone	4—10	8·30	5·88	2·88

[a] For numbering, see text.
[b] Taking the value for the hexane-1,5-diol as 3·0.
[c] Accuracy lowered by small sample size.



SCHEME

in the C-9 region (our numbering) of (I), and demonstrates the formation of (I) from an intact fatty acid (a route which, of course, also explains the pattern of acetate-derived labelling). The ratio of "direct" to "indirect" labelling at C-9 in this case is *ca.* 300%; in the experiment with a longer incubation period, for which equally detailed results were obtained but are not presented here, the same general pattern was revealed but the level of "direct" labelling, though still significant, was relatively lower (*ca.* 50% only) as expected.

From a suitably unsaturated C_{16}-fatty acid, it is possible to envisage oxidative cyclisations leading specifically to (I), *e.g.*, as in the Scheme. The oxidation steps required are analogous to reactions leading from arachidonic acid to the prostaglandins.[5] There are parallels for oxygenation of a fatty acid at the penultimate carbon, and a scheme involving formation of the lactone ring at a relatively early stage has some stereochemical advantages. Details apart, the demonstration of an overall conversion of palmitic acid in (I) also raises the question of corresponding transformations of C_{16}- and C_{18}-acids into other metabolites of the same structural type (some produced by related fungi) such as curvularin (III), zearalenone (IV), *etc.*, and also to such co-metabolites of (I) as palitantin (V).

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[1] R. G. Coombe, P. S. Foss, and T. R. Watson, *Chem. Comm.*, 1967, 1229.
[2] H. P. Sigg, *Helv. Chim. Acta*, 1964, **47**, 1401.
[3] J. Fuska and L. Kukr, *Folia Microbiologica*, 1965, **10**, 357.
[4] J. D. Bu'Lock, G. N. Smith, and C. T. Bedford, *J. Chem. Soc.*, 1962, 1405.
[5] S. Bergstrom, *Science*, 1968, **157**, 382.