The Biosynthesis of Brefeldin A

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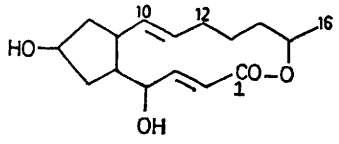
Summary In contrast to the observation that palmitate acts as a specific precursor of brefeldin A (**1**) in *P. cyaneum*, feeding [16-^{14}C]palmitate to *P. lilacinum* gave uniformly labelled brefeldin A.

BU'LOCK and CLAY have reported[1] that one biosynthetic pathway to brefeldin A (**1**) in *Penicillium cyaneum* involved incorporation of intact molecules of [9-^{14}C]palmitic acid. Our work on microbial transformations[2] with a strain of *P. lilacinum* Thom. (IMI 184496), which was at that time a prolific producer of brefeldin A, appeared to be incompatible with such a biosynthetic route, so we have now examined the biosynthesis of brefeldin A in *P. lilacinum*.

Sodium [16-^{14}C]palmitate was fed as an aqueous solution to shake cultures of *P. lilacinum* at 48—95 hours after inoculation and the brefeldin A was isolated 10—12 hours later. The incorporations obtained were much lower (0·05—0·54%) than that reported for [9-^{14}C]palmitate and *P. cyaneum*,[1] but were not unexpected if labelling occurred *via* β-oxidation of the [16-^{14}C]palmitic acid to [2-^{14}C]acetic acid followed by normal polyketide biosynthesis.[3] Oxidation of the labelled brefeldin A by the Kuhn-Roth procedure gave C-15 and C-16 (isolated as *p*-bromophenacyl acetate), and degradation of diacetylbrefeldin A by the method of Sigg[3] afforded C-11 to C-16 as hexane-1,5-diol bis-*p*-nitrobenzoate. The results obtained (Table) show that under our experimental conditions in *P. lilacinum*, the



(1)

TABLE

Degradation of brefeldin A[a]

	Carbon atoms	(d. mmol^{-1} 100 s^{-1}) × 10^{-3} 1	2	% Label 1	2
Brefeldin A	1—16	87·0	17·68	100	100
p-bromophenylacetate	15—16	9·66	2·94	11·2	16·6 (12·5)[b]
Diacetylbrefeldin A	1—16	89·3	8·45[c]	100	100
Hexane-1,5-diol bis-*p*-nitrobenzoate	11—16	32·0	2·78	35·8	32·9 (37·5)

[a] Recently, *P. lilacinum* has given poor yields of brefeldin which have prevented further biosynthetic work with this organism. [b] Figures in parentheses show the value expected from acetate labelled brefeldin A. [c] Diluted with unlabelled brefeldin A before acetylation.

brefeldin A was essentially uniformly labelled and was therefore, not derived from intact palmitate units, but presumably *via* acetic acid. It would appear, therefore, that further examination of the biosynthesis of brefeldin A by *P. cyaneum* is desirable and that the biosyntheses of other mould metabolites such as palitantin and curvularin, which it has been suggested[1,4] may be derived from long chain fatty acids, should be investigated experimentally.

Added in proof: Dr. J. D. Bu'Lock has informed us that studies by Dr. G. N. Smith and Mr. P. Page at the University of Manchester on the metabolism of various labelled fatty acids by *Penicillium brefeldianum* lead to the same conclusion, and that he wishes to withdraw the conclusions in his earlier publication. A full account of the work is in preparation.

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[1] J. D. Bu'Lock and P. T. Clay, *Chem. Comm.*, 1969, 237.
[2] B. E. Cross and P. Hendley, unpublished work.
[3] U. Handschin, H. P. Sigg, and Ch. Tamm, *Helv. Chim. Acta*, 1968, **51**, 1943; R. G. Coombe, P. S. Foss, and T. R. Watson, *Chem. Comm.*, 1967, 1229.
[4] *E.g.*, W. B. Turner, 'Fungal Metabolites,' Academic Press, New York, 1971, p. 72; T. Williams, A. Stempel, R. H. Evans, A. Jacoby, and J. W. Westley, *Experientia*, 1973, **29**, 257.