Biosynthetic Incorporation of Stereoselectively Labelled [β-^{3}H]Tyrosine into Mycelianamide

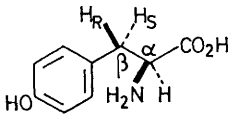
By GORDON W. KIRBY* and SUBRAMANIAN NARAYANASWAMI
(*Department of Chemistry, University of Glasgow, Glasgow* G12 8QQ)

Summary Tyrosine is incorporated, in *Penicillium griseofulvum*, into the dehydro-amino-acid unit of mycelianamide with high retention of the (*pro-R*)- and complete loss of the (*pro-S*)-β-methylene hydrogen atom.

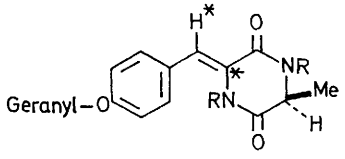
THE metabolism of aromatic amino-acids can involve removal of one of the β-methylene hydrogens by, for example, hydroxylation, alkylation, αβ-elimination, or αβ-desaturation. As part of a general study of the stereochemistry of these processes[1] we have examined the steric course of formal desaturation involved in the incorporation of tyrosine (**1**) into mycelianamide[2] (**2**; R = OH).

Various specimens of tyrosine (see Table), labelled with ^{3}H in the methylene group and with ^{14}C elsewhere, were

administered to cultures of *Penicillium griseofulvum*. After 47 days, mycelianamide was isolated and recrystallised to constant activity. Conversion into deoxymycelianamide (**2**; R = H) caused no significant change in molar activity. The stereoselectively tritiated (*βR*)-[*β*-^{3}H]tyrosine used in these experiments was known[1] to contain both the (*βR*)- (83%) and (*βS*)-form (17%). Conversely, the (*βS*)-[*β*-^{3}H]-tyrosine contained (*βS*)- (87%) and (*βR*)-labelled (13%) species. The results (Experiments 1 and 2 in the Table) showed that formation of the *αβ*-double bond in mycelianamide involved high retention (93%) of (*βR*)-tritium and essentially complete loss of (*βS*)-tritium. In confirmation, the incorporation of non-stereoselectively labelled tyrosine (Experiment 3) was consistent with retention (88%) of (*βR*)- and complete loss of (*βS*)-tritium. A final experiment (Experiment 4), using L-(*βR*)-[*β*-^{3}H]tyrosine mixed with uniformly ^{14}C-labelled L-tyrosine, confirmed the high retention (93%) of (*βR*)-tritium and left little doubt (see later) that biosynthesis involved incorporation of an intact tyrosine carbon skeleton into the metabolite. It is clear that formation of the dehydroamino-acid unit of mycelianamide is subject to stereochemical and, presumably, enzymic control and may be regarded formally as requiring *cis* removal of hydrogen from the L-form of tyrosine. The small loss (7—12%) of (*βR*)-tritium may arise from a stereospecific exchange process like that observed[3] with phenylalanine in *Trichoderma viride*.



(1)



(2)

The labelling pattern of biosynthetic mycelianamide was determined as follows. Deoxymycelianamide from Experiment 3 was reductively cleaved with hydriodic acid under reflux containing red phosphorus to yield DL-tyrosine with retention of ^{3}H (94%) and ^{14}C (98%). The tyrosine was methylated with dimethyl sulphate and alkali and the mixture heated to induce Hofmann elimination. The total product was oxidised directly with potassium permanganate to give 4-methoxybenzoic acid lacking both ^{3}H and ^{14}C. Decarboxylation of the tyrosine in hot diphenylamine gave tyramine with retention of ^{3}H (94%) and ^{14}C (105%). Thus the expected labelling pattern [asterisks in (**2**)] was established. Similarly, deoxymycelianamide from Experiment 4 gave tyrosine (96% ^{3}H and 99% ^{14}C retained) and thence 4-methoxybenzoic acid (79% ^{14}C and a negligible amount of ^{3}H retained). The ^{14}C content of this acid agrees well with that expected (78%) for a uniformly labelled precursor. Moreover, degradation of the precursor itself gave 4-methoxybenzoic acid with a similar retention (77%) of ^{14}C.

TABLE

Incorporation[a] *of tyrosine* (**1**) *into mycelianamide* (**2**; R = OH)

Experiment	Labelling pattern[b] in (**1**)	Retention (%) of ^{3}H in (**2**; R=OH)	(**2**; R=H)
1	DL-(*βR*)-[*β*-^{3}H,*α*-^{14}C]	76·5	77·3
2	DL-(*βS*)-[*β*-^{3}H,*α*-^{14}C]	15·5	15·5
3	DL-(*βRS*)-[*β*-^{3}H,*α*-^{14}C]	44·2	44·2
4	L-(*βR*)-[*β*-^{3}H,*U*-^{14}C]	77·2	77·8

[a] Incorporations were in the range 0·5—3·3%. [b] ^{3}H:^{14}C ratios were typically 6·5:1.

Birch *et al.*[2,4] studied the biosynthesis of the geranyl unit of mycelianamide and reported, without details, the incorporation of tyrosine into this metabolite.

We thank the S.R.C. for financial support.

(*Received*, 30*th March* 1973; *Com.* 443.)

[1] G. W. Kirby and J. Michael, *J.C.S. Perkin I*, 1973, 115.
[2] A. J. Birch, R. A. Massey-Westropp, and R. W. Rickards, *J. Chem. Soc.*, 1956, 3717; K. W. Blake and P. G. Sammes, *J. Chem. Soc.* (*C*), 1970, 980; R. F. C. Brown and G. V. Meehan, *Austral. J. Chem.*, 1968, **21**, 1581.
[3] J. D. Bu'Lock, A. P. Ryles, N. Johns, and G. W. Kirby, *J.C.S. Chem. Comm.*, 1972, 100; recent unpublished work has shown that exchange of (*βR*)- not (*βS*)-hydrogen occurs in this system.
[4] A. J. Birch, R. A. Massey-Westropp, R. J. English, and H. Smith, *J. Chem. Soc.*, 1958, 369; A. J. Birch, M. Kocor, N. Sheppard, and J. Winter, *J. Chem. Soc.*, 1962, 1502.