BIOSYNTHESIS OF DITHIADIKETOPIPERAZINE ANTIBIOTICS: COMPARISON OF POSSIBLE AROMATIC AMINO ACID PRECURSORS

D. R. Brannon, J. A. Mabe, B. B. Molloy, and W. A. Day
The Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, Indiana 46206

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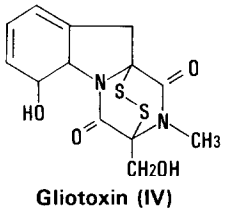
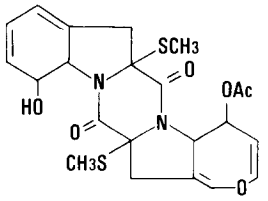
Summary: Phenylalanine-^{14}C-3 was shown to be a more efficient precursor than m-tyrosine-^{14}C-2 for the production of gliotoxin by Penicillium terlikowskii or Trichoderma viride, as well as for production of bisdethio-di(methylthio)-acetylaranotin by Arachniotus aureus. All of the aromatic and both of the methylene deuteriums of phenylalanine-d_8 were retained upon incorporation into acetylaranotin by Aspergillus terreus.

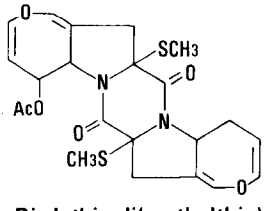
The aranotins, a novel oxepin ring-containing class of diketopiperazines, were independently obtained in two laboratories from different fungi (4,6,7,8). Structural similarities between aranotins I-III and the fungal metabolite gliotoxin (IV) suggested a similar biosynthetic pathway. Preliminary experiments indicated (7) an aromatic amino acid origin for the 7-member ether ring of the aranotins. We now report a study of the incorporation of a variety of aromatic amino acids into the aranotin series and gliotoxin, which supports our proposed (7,8) mechanism involving arene oxide intermediates.



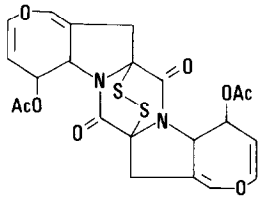
Gliotoxin (IV)



Bisdethio-di(methylthio)-apoaranotin (I)



Bisdethio-di(methylthio)-acetylaranotin "BDA" (III)



Acetylaranotin (II)

Incubation of DL-m-tyrosine-^{14}C-2(m-tyr), DL-phenylalanine-^{14}C-3(ph al) and 3,5-dihydroxyphenylalanine-^{14}C-2(3,5-dopa) with metabolizing cultures of Arachniotus aureus resulted in the intracellular accumulation of label as shown in Table 1. The rate of accumulation of label from m-tyrosine was comparable to that of phenylalanine.

TABLE 1

Intracellular Accumulation of C-14 from Incubation of Labeled Amino Acids with A. aureus*

Duration of substrate incubation	Intracellular accumulation of label, CPM		
	DL-ph al-^{14}C-3 10.0 μCi, 3.8 mg	DL-m-tyr-^{14}C-2 10.5 μCi, 3.8 mg	DL-3,5-dopa-^{14}C-2 10.5 μCi, 3.8 mg
10 sec	2297	1900	499
20 sec	1730	1853	410
40 sec	2430	2394	609
1 min	4327	3941	528
2 min	4341	5100	754
5 min	9218	8717	606
1 hr	80840	80906	3565

*Fermentation conditions for Arachniotus aureus, NRRL 3205, have been previously described (7).

TABLE 2

Incorporation of C-14 from Labeled Amino Acids into Gliotoxin (IV) by T. viride*

Substrate	Amount added		Incubation period (hour from inoculation)	Incorporation of label into IV (%)
	mg	μCi		
DL-m-tyr-^{14}C-2	0.706	2.1	48-72	0.04
DL-ph al-^{14}C-3	0.34	10.0	48-72	2.60

*Trichoderma viride, NRRL 1828, was grown in 50 ml of a synthetic sucrose medium (5).

A comparison of the efficiency of incorporation of DL-m-tyrosine-^{14}C-2 and DL-phenylalanine-^{14}C-3 into gliotoxin by Trichoderma viride is shown in Table 2. Phenylalanine repeatedly gave a higher percentage of incorporation than did m-tyrosine.

Table 3 shows the incorporation of label from amino acids into III by A. aureus, and into IV by Penicillium terlikowskii. Like gliotoxin, bisdethio-di(methylthio)-acetylaranotin (BDA, III) was precursed more efficiently by phenylalanine than by m-tyrosine.

TABLE 3

Incorporation of C-14 from Labeled Amino Acids into III by A. aureus[1] and IV by P. terlikowskii[2]

Substrate	Amount added mg	Amount added uCi	Incubation period (hour from inoculation)		Incorporation of label (%) into III[1]	Incorporation of label (%) into IV[2]
DL-m-tyr-^{14}C-2	0.750	2.040	72-96[1]	48-72[2]	0.38	0.37
	0.075	0.204	72-92[1]	48-72[2]	0.46	0.12
DL-ph al-^{14}C-3	0.10	10.0	72.96[1]	48-72[2]	6.64	9.10
DL-ph al-^{14}C-3 plus DL-ph al	0.10 15.00	10.0 0.0	72-96[1]		0.79	--
DL-3,5-dopa-^{14}C-2	0.750	1.680	72-96[1]	48-72[2]	0.18	0.01
	0.075	0.168	72-96[1]	48-72[2]	0.17	0.00

[1]Fermentation conditions for Arachniotus aureus, NRRL 3205, have been previously described (7).

[2]Penicillium terlikowskii, NRRL 2067, was grown in 50 ml of a synthetic dextrose medium.

Table 4 shows the incorporation of label into III when DL-phenylalanine-^{14}C-3 and DL-phenylalanine-^{3}H-ortho were added as a mixture to A. aureus.

TABLE 4

Comparison of Incorporation of ^{14}C-3 and ^{3}H-ortho Labels of Phenylalanine into III by A. aureus[1]

Substrate	Amount Added mg	Amount Added μCi	Incubation period (hour from inoculation)	Incorporation of label into III (%) ^{14}C	Incorporation of label into III (%) ^{3}H
DL-ph al-^{14}C-3 plus DL-ph al-^{3}H-ortho	0.34 0.02	10.0 50.0	72-96	7.04[2]	5.67[2]

[1]Fermentation conditions for Arachniotus aureus have been previously described (7).

[2]Average of duplicate experiments.

Table 5 shows the mass spectra of acetylaranotin (II) and the isotopic mixture of acetylaranotin obtained from incubation of DL-phenylalanine-d_8 with Aspergillus terreus. Both spectra were obtained under identical instrument conditions.

TABLE 5

Mass Spectral Data of Aranotin II and the Deuterated Aranotin II Mixture Obtained by Precursing A. terreus* with L-phenylalanine-d_8

m/e	Relative abundance of molecular ions: Deuterated aranotin II mixture	Aranotin II
440 (II-S_2)	32	55
441	9	15
446	10	1
447 (II-S_2+d_7)	31	0
448	8	0
453	6	0
454 (II-S_2+d_{14})	12	0
455	2	0

*Aspergillus terreus, Lilly A27160.3, an isolate of NRRL 3319, was grown in 50 ml of a glucose medium. L-phenylalanine-d_8 (Merck & Co., Rahway, New Jersey) was added to three flasks, each receiving five additions of 1.6 mg.

Conclusions

Under all conditions examined, DL-phenylalanine was found to be a more efficient precursor of aranotin III than was DL-m-tyrosine.

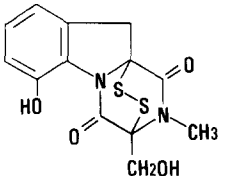
At the time this work was undertaken, it had been claimed that m-tyrosine was the direct precursor to gliotoxin (IV) (9,10). This viewpoint was difficult to reconcile with the superiority of phenylalanine in precursing aranotin III, since A. aureus also produces the hydroxycyclohexadiene-containing apoaranotin (I), implying that the initial steps in the formation of the oxepin ring were similar to those leading to the hydroxy-cyclohexadiene-containing gliotoxin. Impermeability of A. aureus to exogeneous m-tyrosine might have been one explanation to this apparent anamoly; however, the intracellular accumulation of C-14 in A. aureus from labeled m-tyrosine and phenylalanine were virtually parallel. In contrast, label from 3,5-dopa was not accumulated by A. aureus.

In subsequent experiments, the relative efficiencies of incorporation of phenylalanine and m-tyrosine into gliotoxin were examined. Phenylalanine was found to be more efficiently incorporated into gliotoxin by T. viride in a synthetic medium, and by P. terlikowskii in a complex medium, than was m-tyrosine. These results agree with a recent finding by Bu'Lock and Ryles (3) that phenylalanine, but not o- or m- tyrosine or 2,3-dopa, is incorporated into gliotoxin by T. viride.

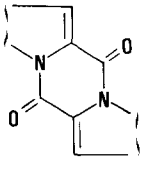
A mechanism for the formation of gliotoxin and the aranotins has been proposed involving a phenylalanine-2,3-epoxide intermediate (7,8). Contrary to such a mechanism is a report that the aromatic 2,3-dehydrogliotoxin (VIII) can be converted by

Penicillium terlikowskii into gliotoxin (1). The incorporation of label into aranotin III from DL-phenylalanine-^{3}H-ortho, to the extent of 80% of the incorporation of label from DL-phenylalanine-^{14}C-3, negates a biosynthetic route involving the latter mechanism. This conclusion is substantiated by the retention of all five ring deuteriums and both C-3 methylene deuteriums when L-phenylalanine-d_8 was used to precurse acetyl-aranotin. This experiment was conducted with A. terreus and not A. aureus because of the complexity of the aranotin mixture obtained from the latter culture. This result is in agreement with the conclusion of Bu'Lock and Ryles (3), that gliotoxin is formed from phenylalanine by a route in which all of the aromatic hydrogens of phenylalanine are retained. Thus the intermediacy of a phenylalanine-2,3-epoxide (7,8), common to both gliotoxin and aranotin formation is attractive.

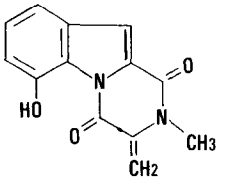
In addition, the retention of the two C-3 methylene deuteriums during the conversion of L-phenylalanine-d_8 to acetyl-aranotin indicates that introduction of the disulfide bridge could not have arisen by addition of sulfur across the diene system bonds of an intermediate of partial structure IX. A compound of structure X has been isolated from P. terlikowskii, and its intermediacy in the pathway to gliotoxin has been proposed (2).



VIII



IX



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