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SHORT COMMUNICATION

STUDIES ON THE BIOSYNTHESIS OF LYCOMARASMIN

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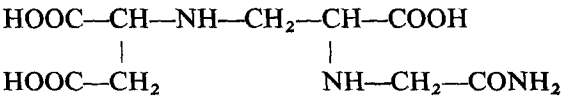
LYCOMARASMIN, the structure of which was determined in 1962,[1] is a phytotoxin produced by various strains of *Fusarium*. At least two other wilt toxins are produced by *Fusarium oxysporum, f. lycopersici,* one of which is fusaric acid.[2] The biosynthesis of this pyridine alkaloid has been established.[3,4]

The structure of lycomarasmin (I) indicates that at least some parts of the molecule should be derived from simple amino acids or their deaminated keto analogs. Sets of different amino acids may be postulated as possible precursors depending on how the molecule is subdivided into its apparent structural units. Such a subdivision of the toxin finds its basis in the fact that acid hydrolysis of lycomarasmin gives rise to aspartic acid, pyruvic acid and glycine.[1]



(I) Lycomarasmin

Stock cultures of the fungus were easily maintained on potato-dextrose agar slants. For mass cultures, small portions of mycelium were aseptically transferred into 2 l. Erlenmeyer flasks containing 500 ml modified Richards' medium. The still cultures were maintained at 25° in the dark for 7 weeks after which 10 μCi ^{14}C-labelled amino acid (glycine, serine, aspartic acid and alanine) was added per flask. The cultures were killed after 24 hr contact with the labelled compounds. Crystalline lycomarasmin was isolated using a published isolation procedure.[1] The specific activity of isolated toxin obtained from the different feeding experiments and the specific activity of the hydrolysis products of labelled toxin are shown in Tables 1 and 2.

The results in Table 1 strongly indicate that all the administered ^{14}C-labelled amino acids serve as relatively efficient precursors. The label distribution observed in Table 2 can be readily explained in terms of known enzymatic interconversions of the administered labelled amino acids. It seems apparent that glycine may be incorporated as such or may be deaminated and the generated glyoxylate in turn condense with serine. The label distribution when ^{14}C-labelled glycine and serine are administered might be expected on the basis of the

[1] E. HARDEGGER, P. LEICHTI, L. M. JACKMAN, A. BOLLER and P. A. PLATTNER, *Helv. Chem. Acta* **46**, 60 (1963).
[2] T. YABUTA, *J. Agric. Chem. Soc. Japan* **10**, 1059 (1934).
[3] R. D. HILL, A. M. UNRAU and D. T. CANVIN, *Can. J. Chem.* **44**, 2077 (1966).
[4] L. C. VINING, *Can. J. Biochem.* **46**, 1293 (1968).

TABLE 1. SPECIFIC ACTIVITY OF LYCOMARASMIN

Amino acid*	Specific activity of Lycomarasmin (counts/min/mM $\times 10^{-4}$)
Glycine-U-^{14}C	3·34
L-Serine-U-^{14}C	3·25
DL-Aspartic acid-4-^{14}C	1·67
DL-Alanine-1-^{14}C	2·01

* 50 μCi of each fed.

glycine-serine interconversion via the biological aldol-retro-aldol reaction and both compounds can eventually be incorporated into aspartic acid. Aspartate can apparently serve as the four-carbon source and the observed label incorporation into the three carbon fragment could readily occur via fumarate ($C_4 \equiv C_1$) → malate → oxalacetate → phosphoenol pyruvate → 3-hydroxy pyruvate → serine. In the case of alanine, incorporation into aspartate (the four carbon fragment) could occur via pyruvate → oxalacetate → fumarate. Oxalacetate thus derived by carboxylation may give rise to phosphoenolpyruvate which can eventually give rise to 3-hydroxypyruvate as indicated above. The low incorporation of label into the two carbon fragment (glycine) when labelled aspartate and alanine were administered is of

TABLE 2. SPECIFIC ACTIVITY OF HYDROLYSIS PRODUCTS OF ^{14}C-LABELLED LYCOMARASMIN

	Hydrolysis products (counts/min/mM $\times 10^{-4}$)		
Labelled Amino acid admin.	Glycine	Aspartic acid	Pyruvic acid (as 2,4-DNPH)
Glycine-U-^{14}C	1·04	0·37	1·54
L-Serine-U-^{14}C	0·97	0·44	1·44
DL-Aspartic acid-4-^{14}C	0·07	1·12	0·41
DL-Alanine-1-^{14}C	0·05	0·99	0·97

considerable interest. This observation at least tentatively suggests that the conversion of these compounds into a suitable 3-carbon source does not proceed past 3-hydroxypyruvate since conversion of this compound to serine should then lead to incorporation of label into C_1 of glycine. This transformation was observed as would be expected when labelled glycine and serine were administered. The incorporation pattern observed for aspartic acid and alanine tentatively indicates that a condensation of hydroxypyruvate and glycine may be involved in the biogenesis of the 2-C and 3-C portions of the toxin. Investigations are in progress to determine the sequence of events in the overall condensation and the nature of the immediate precursors involved.

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