Production of Gliotoxin by *Aspergillus fumigatus* mut. *helvola* Yuill

IN the Research Items in NATURE of April 29, an account is given of the production of antibiotics by *Aspergillus fumigatus*. To supplement this annotation we submit this short account of our own work with the mould *Aspergillus fumigatus* mut. *helvola* Yuill. An earlier publication[1] has described the isolation of helvolic acid from 2–3-week cultures of this mould grown at 25° C. on a medium containing mineral salts and 4 per cent of glucose.

Discrepancies between the antibacterial activity of the culture medium, particularly during the first few days of growth, and the yield of helvolic acid obtained, suggested the presence of a second antibacterial substance. It was found that whereas the antibacterial activity of a 20–22-day culture is scarcely diminished at all by maintaining it at *p*H 10 for 5 hours at 37° C., this treatment completely destroys the activity of 3–4-day cultures. Since helvolic acid itself is stable at *p*H 10 under these conditions, it is clear that in the early stages of growth a second antibiotic, sensitive to very dilute alkali, is produced.

Investigation showed that the activity of the medium reaches a maximum after 8–9 days, then drops somewhat to reach a minimum after 16–17 days, after which it again rises to reach a fairly constant value after 20–22 days. At this time the mould is usually harvested for the isolation of helvolic acid. To isolate the second antibiotic, 5–6 day cultures are brought to *p*H 10 and immediately extracted thrice with equal volumes of chloroform, the total volume of chloroform being equal to the volume of medium being extracted. The chloroform extract is distilled under reduced pressure and the solid residue crystallized several times from hot alcohol. This process yields long colourless needles of the second antibacterial substance, the weight obtained being, roughly, 30 mgm. per litre of culture medium.

For the isolation of helvolic acid in this laboratory a magnesia column is used[1] and this is eluted with hot water. Under these conditions, the alkali-sensitive material is destroyed. We have identified the second antibiotic as one already described, gliotoxin, first obtained[2] as a metabolic product of the mould *Gliocladium fimbriatum* Gilman and Abbott. Our identification is based primarily on the data of Johnson, Bruce and Dutcher[3], who have investigated the properties of gliotoxin in considerable detail.

Property	Gliotoxin	Data of Glister and Williams
Melting point*	221° C.	218-220° C.
Optical activity	$[\alpha]_D^{25} = -255° \pm 15°$ (0·1% in $CHCl_3$) $[\alpha]_D^{25} = -290° \pm 10°$ (0·08% in C_2H_5OH)	$[\alpha]_D^{20} = -254°$ (0·6% in $CHCl_3$)
% Carbon†	48·08	49·9
% Hydrogen	4·96	4·4
% Nitrogen	8·15	9·5
% Sulphur	19·29	19·3

* In common with other authors we find that this value is obtained only if heating is very rapid.
† Analyses by Weiler and Strauss. Material dried at 50° C. *in vacuo*.

The molecular weight determined by an X-ray method (D. Crowfoot and B. W. Rogers-Low) is 330 ± 8. Johnson *et al.*[3] give 314 as their best value. The absorption spectrum, examined by Dr. E. R. Holiday, is consistent with the findings of these workers[3] that gliotoxin is an indole derivative.

The antibacterial activity of our material has been determined by Dr. M. A. Jennings, of this Department. In serial dilution tests, in which one drop of a 16-hour bacterial culture, diluted 1 : 1,000, was added to 5 c.c. quantities of nutrient broth containing diminishing amounts of gliotoxin, the minimum concentrations required to inhibit growth completely were :

Staph. aureus	1 : 360,000
S. Typhi	1 : 45,000
Bact. coli	>1 : 45,000

The sensitivity depends very much on the size of inoculum; for example, when the inoculum of *Staph. aureus* was increased a thousandfold, the minimum growth-inhibiting concentration was increased eightfold.

In the original publication[1] on helvolic acid, reference was made to fumigacin, an antibacterial substance obtained by Waksman and his collaborators[4] from *Aspergillus fumigatus* (Strain W84). The data then published were sufficient to establish that fumigacin and helvolic acid were not the same. In particular, fumigacin contains 3·7 per cent nitrogen (helvolic acid contains no nitrogen), 62·7 per cent carbon (helvolic acid, 69·1 per cent) and melts at 185–87° C. (helvolic acid, 212° C.). During the course of the present work, however, it has been shown[5] that fumigacin is a mixture of helvolic acid and gliotoxin. In view of this it seems rational to replace the term fumigacin, relating to a mixture of antibiotics, by the separate names gliotoxin and helvolic acid, which refer to chemical individuals. In this laboratory we have been able to obtain gliotoxin from 5–7-day cultures of a strain of *Aspergillus fumigatus* Fres. (Lister No. 982) kindly supplied by Dr. W. H. Wilkins, Mycology Laboratory, University Department of Botany. The mould was grown at 25° C. on Czapek-Dox plus 2 per cent glucose.

Shortly before submitting this note for publication, it was reported that gliotoxin has been obtained from a Penicillium as yet unidentified[6].

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Sir William Dunn School of Pathology,
Oxford. May 1.

[1] Chain, Florey, Jennings and Williams, *Brit. J. Exp. Path.*, **24**, 108 (1943).
[2] Weindling and Emerson, *Phytopath.*, **26**, 1068 (1936).
[3] Johnson, Bruce and Dutcher, *J. Amer. Chem. Soc.*, **65**, 2005 (1943).
[4] Waksman, Horning and Spencer, *J. Bact.*, **45**, 233 (1943).
[5] Menzel, Wintersteiner and Hoogerheide, *J. Biol. Chem.*, **152**, 419 (1944).
[6] Johnson, McCrone and Bruce, *J. Amer. Chem. Soc.*, **66**, 501 (1944).

X-Ray Crystallography of Gliotoxin

WE have examined a specimen of gliotoxin prepared by G. A. Glister and T. I. Williams, as above. The crystals are four- and six-sided monoclinic plates elongated along [010], and our evidence on their morphology and optic character agrees very well with the measurements of Dr. W. C. McCrone[1,2].

The crystals gave good X-ray photographs from which the following data were obtained : $a = 18·74$ A., $b = 7·59$ A., $c = 10·36$ A., $\beta = 80°$, correct to about ± 1 per cent ; space group $P2$. Density = 1·543 ± 0·01.

Since the space group requires at least two molecules in the unit cell, the crystallographic molecular