THE ISOLATION OF GLIOTOXIN AND FUMIGACIN FROM CULTURE FILTRATES OF ASPERGILLUS FUMIGATUS

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Some time ago Waksman, Horning, and Spencer (1) reported the isolation of a new antibiotic agent, fumigacin, from culture filtrates of Aspergillus fumigatus. The authors recorded the melting point (185–187°) and nitrogen content (3.7 per cent) of their crystalline product, but otherwise confined themselves to a description of its biological properties. In a study aiming at a more complete chemical characterization of this compound we abandoned the charcoal adsorption process of Waksman et al. in favor of direct extraction of the acidified filtrate with ether or other immiscible solvents. It soon became apparent that two antibiotically active entities were present in these extracts: (1) a neutral, nitrogen- and sulfur-containing compound melting at 195° which was identified as gliotoxin, previously isolated by Weindling and Emerson (2) from culture filtrates of Gliocladium fimbriatum; (2) a weakly acidic substance melting at 220° which is free of nitrogen and sulfur, and unquestionably represents the fumigacin of Waksman in pure form.

Fumigacin is levorotatory ([α]ₚ = −132°). Its ultraviolet spectrum exhibits no maximum but merely strong end-absorption. Marked and so far unexplained inconsistencies in the molecular weight determinations by the Rast procedure and titrations for equivalent weight made it precarious to assign a definite empirical formula to the compound. Nor could this uncertainty be entirely resolved by the preparation of derivatives, although the data given by the latter narrowed the range for the molecular size. Fumigacin readily yields a methyl ester with diazomethane. The analytical figures for this ester, especially its repeatedly determined methoxyl content, indicated the composition C₃₀H₄₀–₄₂O₇; consequently fumigacin itself would be C₂₉H₃₈–₄₀O₇, which is compatible with its carbon and hydrogen content. However, analyses of a crystalline silver salt were in close agreement with the theoretical values for C₂₉H₃₈O₆Ag. Other metal salts could not be obtained in pure condition. Various other derivatives were prepared for the purpose of characterizing the remaining oxygen atoms, but here again the analytical results were too ambiguous to permit a choice between the two formulae now considered most likely on the basis of the evidence given above. We ascribe these difficulties to the fact that the
solubility properties of these derivatives are so similar to those of fumigacin that, at least in some cases, contamination with the latter may have occurred. Much as a repetition of these experiments seems desirable, we are compelled to present our findings as they now stand because with the exhaustion of our supply of fumigacin this study had to be discontinued for the sake of more pressing matters.

Since fumigacin has no phenolic or enolic properties, 2 oxygen atoms must be present in the form of a carboxyl group. Its antibiotic activity is quickly abolished when it is dissolved in caustic alkali. This change is accompanied by a rapid rise of the levorotation and results, under suitable conditions, in the precipitation of a crystalline sodium salt. Back titration showed that a second acidic group, presumably formed by the opening of a lactone ring, had been liberated, but the corresponding free acid could not be obtained in crystalline form. Acetyl determinations on fumigacin, though quantitatively unsatisfactory, gave evidence for the presence of one such group. On the other hand, the presence of a free, readily esterifiable hydroxyl group must be considered doubtful. Attempts to acetylate the compound with acetic anhydride and pyridine under a variety of conditions, or with boiling acetic anhydride alone, led to mixtures of variable composition which resembled the starting material in respect to solubility and melting point range. Treatment with benzoyl chloride or p-nitrobenzoyl chloride in pyridine failed to yield crystallizable products. More satisfactory results were obtained with carbonyl reagents. Though the reactions with phenylhydrazine and its nitro derivatives were negative, we could secure an oxime and a semicarbazone, both in crystalline form. The nitrogen content of these derivatives, though it did not correlate too closely with the two formulae under consideration, at least assured the presence of one carbonyl group in the molecule. That this group must be ketonic in nature follows from the positive Zimmermann test and the absence of aldehydic properties.

Fumigacin reacts rapidly with bromine and with permanganate either in sodium carbonate or acetone solution. However, no hydrogen uptake was observed in the presence of a platinum catalyst, and starting material was recovered from the reaction. A crystalline bromine derivative (m.p. 178°) was obtained by reaction with approximately 2 atoms of bromine in chloroform, but the analytical data (C 57.93, H 6.53, Br 16.36) could not be reconciled with either of the two formulae considered above.

After completion of this work a paper by Chain, Florey, Jennings, and Williams (3) came to our attention, in which these authors describe the isolation from culture filtrates of Aspergillus fumigatus of an antibiotic substance, designated by them helvolic acid. The properties and com-
The position of this compound and of its methyl ester leave little doubt that it is identical with the fumigacin described here. The only serious point of divergence is the specific rotation, which is given as \(-49.4^\circ\), while our value in the same solvent is \(-132^\circ\). Since our figure has been repeatedly checked, we are inclined to ascribe the discrepancy to a factual or clerical error on the part of the British authors. The formula assigned to helvolic acid on the basis of titration and crystallographic x-ray data is C_{22}H_{44}O_{8}, which is in agreement with the analysis of our silver salt. However, it should be noted that their figure for the Rast molecular weight (510) fits much better with the C_{29} formula which is favored by most of our own evidence. No mention is made by the British authors of the occurrence of a sulfur-containing entity.

The antibacterial potency of fumigacin per mg. is considerably less than that of other antibiotic substances such as penicillin, gramicidin, tyrocidin, and gliotoxin, but it is of the same order as that of aspergillus acid. Whereas the latter compound acts on Gram-negative as well as Gram-positive bacteria, fumigacin is effective primarily against Gram-positive bacteria.

The toxicity of pure fumigacin was found to be considerably less than reported by Waksman et al. This difference may be due to the presence in their preparation of small amounts of gliotoxin, a substance which is considerably more toxic than fumigacin. To judge from the antibacterial efficacy in vitro, subtoxic doses of fumigacin should be capable of inhibiting the growth of sensitive pathogenic bacteria such as *Streptococcus haemolyticus* or *Staphylococcus aureus* also in vivo. Since peptones, serum, or whole blood does not interfere seriously with its bacteriostatic action in vitro, the prerequisites for employing fumigacin as an effective therapeutic agent exist. Results obtained in protection experiments gave some slight promise in this respect. By using amounts close to the maximal tolerable dose and by proper timing of the treatment, protection was afforded to about 50 per cent of mice infected with *Streptococcus haemolyticus*. All the above observations on the biological properties of fumigacin are substantially in agreement with those of Chain et al. on helvolic acid, thus strengthening the chemical evidence for the identity of the two compounds.

Our yields of the pure compounds as well as specific inactivation of the gliotoxin moiety in the culture filtrate by chemical means indicated that this substance, and not fumigacin, accounts for most of the antibiotic activity produced by the mold in Czapek-Dox medium. An inquiry into the composition of the crystals yielded by the method of Waksman therefore seemed in order. Through the kind cooperation of Dr. Waksman we had an opportunity to examine his own crystalline material as well as the
active, crystallizable fraction prepared from charcoal adsorbates which he furnished to us.\textsuperscript{1} Pure fumigacin could be readily isolated from these materials, and although we were unable to secure the gliotoxin moiety in crystalline form, the evidence presented in the experimental part leaves little doubt that substantial amounts of the latter compound were likewise present. Apparently the charcoal procedure yields in the active fraction, besides the two active constituents, some contaminating substances, most likely decomposition products of gliotoxin, which impede the crystallization of the latter compound. It should be mentioned that the same difficulty was encountered also in our own procedure of isolation when brown sugar was substituted for glucose in the culture medium, although this measure about doubled the yield of crystalline fumigacin.

\textit{Aspergillus fumigatus} is by no means unique among molds in its capacity to produce two compounds entirely unrelated chemically, both possessing strong antibiotic activity. In the few instances thoroughly investigated generally one of the antibiotic factors greatly preponderated over the other, depending on the culture medium and the conditions of growth. Thus \textit{Aspergillus flavus} when grown on the surface of a tryptone medium will produce large amounts of aspergillic acid (4), but very little of the penicillin-like substance which it forms in submerged culture (5), while the opposite is true when a modified Czapek-Dox medium with certain adjuvant substances is used for submerged cultivation. With \textit{A. fumigatus}, on the other hand, we have not been able to dissociate to any great extent the metabolic processes leading to the production of gliotoxin, on the one hand, and of fumigacin, on the other, except by the obvious means of greatly reducing the inorganic sulfur content of the medium. Though very little gliotoxin is produced in such a medium, the formation of fumigacin is not measurably impaired. It must therefore be concluded that the two processes represent entirely independent metabolic pathways and are in no stage coupled with each other. On the other hand, if the mold is grown in submerged instead of in surface culture, the proportion in which the two compounds are produced is not appreciably altered, but the yields of both are slightly higher. This is in marked contrast to the behavior of \textit{A. flavus} mentioned above. Some change in the ratio does occur, however, when the glucose in the Czapek-Dox medium used for surface cultivation is replaced by brown sugar. The yield of fumigacin is approximately doubled, while gliotoxin production is decreased; as mentioned before, the neutral fraction was in this case contaminated with by-products which prevented the crystallization of gliotoxin. However, the latter difficulty

\textsuperscript{1} We wish to express our sincere thanks to Dr. Waksman for his interest and helpful advice and for the material assistance rendered by furnishing the culture of \textit{Aspergillus fumigatus} and the chemical preparations mentioned.
was not encountered when the mold was grown by submerged cultivation in the brown sugar-containing medium devised for this purpose.

EXPERIMENTAL

Microbiological (J. C. Hoogerheide)

Production and Activity of Mold Filtrates—In the routine production of the mold filtrate for chemical studies, Aspergillus fumigatus (Strain W84*) was grown at 24° in 1 gallon flint bottles each containing 1 liter of ordinary Czapek-Dox medium made up with tap water. Optimal activity was usually reached between the 6th and 8th day of incubation, when the filtrate was harvested and extracted with the shortest possible delay. The pH attained at this point was usually around 6.0.

The filtrate activity was measured by the usual serial dilution technique with Staphylococcus aureus as the test organism. As a rule, the limiting dilution just causing inhibition of bacterial growth was 1:500 when the medium was made up with distilled water, 1:250 when tap water was used.

A more detailed study of the factors governing the production of the two active agents is in progress. However, a few facts are sufficiently established to warrant mention here. Traces of heavy metals appear to play an important rôle. For instance, addition of 1 mg. of zinc sulfate per liter of medium will entirely suppress the production of antibiotic substances without impairing the growth of the mold. Instead, a dark red pigment, possibly the p-benzoquinone derivative fumigatin of Oxford and Raistrick (6), is formed. The lower activity resulting from the use of tap water instead of distilled water in the medium may likewise be ascribed to the presence of small amounts of inhibiting metals. This effect was discovered only after the isolation work had been completed, so that the yields given in the chemical part should not be considered as optimal.

The results described in the following were for the most part obtained in small scale experiments in which tap water was used in the medium. Generally only the total activity of the filtrate was measured, but a few preliminary results obtained later by means of a test which permits differentiation between the gliotoxin and fumigacin moieties are included. The differential method is based on the fact that gliotoxin, which contains a disulfide linkage extremely labile to alkali,2 is completely inactivated by heating at 100° for 5 minutes in 1 per cent sodium bicarbonate solution. Fumigacin, though it likewise loses its activity by similar treatment with

2 We are indebted to Dr. J. D. Dutcher of the Division of Organic Chemistry for advance information concerning the chemical properties and structure of gliotoxin, and for supplying the reference specimen derived from Gliocladium fimbriatum. A detailed account of Dr. Dutcher’s work on the constitution of this compound will be published soon from Cornell University.
sodium carbonate, or in the course of several hours when dissolved in cold caustic alkali, is virtually unaffected by sodium bicarbonate under these conditions. Assays of filtrate samples before and after such treatment in conjunction with the known ratio of the activities of the pure compounds against Staphylococcus aureus (fumigacin = 1, gliotoxin = 6) thus give a rough measure of the ratio in which both active components are present in the filtrate.

In unmodified Czapek-Dox medium more than 90 per cent of the total activity was found to be due to gliotoxin. On the weight for weight basis, therefore, both compounds should be present in approximately equal proportions. (The yields by actual isolation did not reflect this ratio; in fact usually about 5 times more crude gliotoxin than fumigacin were obtained. However, not too much significance should be ascribed to this discrepancy, as the loss of total activity in the extraction and isolation procedure was considerable.) Continued incubation beyond 1 week caused a considerable drop in total activity, which was found to be entirely due to a gradual destruction of gliotoxin, the fumigacin content remaining virtually unchanged.

The substitution of brown sugar for glucose in the medium decreases the maximum total activity attainable. This is caused by diminished production of gliotoxin, which is only partly compensated for by increased formation of fumigacin. The latter observation is borne out by the markedly greater yield of this compound from such filtrates by chemical isolation. This effect of brown sugar may be connected with the fact that the pH is considerably higher, about 7.0, at the time when the maximum titer is reached. Variation in the carbohydrate (glucose or brown sugar) concentration in the medium between 0.5 and 9 per cent had no appreciable influence on the final titer. Sucrose is about equivalent to glucose, but substitution of the latter by lactose, starch, glycerol, mannitol, sodium lactate, and sodium acetate abolished the ability of the mold to produce antibiotic activity, and very low titers only were obtained with maltose, inulin, salcin, and calcium gluconate, though the mold grew abundantly on most of these media. Addition of peptone to the regular glucose-containing medium likewise caused a marked drop in activity.

When the mold was deprived of its principal source of sulfur by substituting in the medium magnesium chloride for magnesium sulfate, the production of fumigacin was normal (as also shown by isolation) but, as was to be expected, very little gliotoxin was formed.

Marked antibiotic activity is produced when the mold is grown in submerged culture, either in an Erlenmeyer flask shaken on a machine or in a tank provided with stirring and aeration devices. The medium most suitable for this purpose is composed as follows: KH$_2$PO$_4$ 0.1 per cent, NaNO$_3$ 0.6 per cent, MgSO$_4$ 0.05 per cent, CaCO$_3$ 1.5 per cent, brown sugar
The optimal titer was reached after 6 days. The proportion of the two active agents was not materially different from that obtained by static culture, though the yield of both was slightly better (isolation).

**Antibacterial Properties of Fumigacin**—Whereas gliotoxin is active against Gram-negative as well as Gram-positive bacteria, fumigacin acts primarily on the latter.

Table I illustrates the inhibitory effect of fumigacin on the growth of several strains of staphylococci and streptococci. The figures for the 4 to 7 hour period give a measure of the bacteriostatic effect, while those in the last column are the concentrations necessary for permanent inhibition and therefore may be taken as indicating the bactericidal efficacy.

The presence of serum in the test medium causes only slight inhibition of the bacteriostatic action.

**Toxicity and Protective Experiments**—The L.D. 50 for 20 gm. mice, injected intraperitoneally, was found to be 8 mg., or 400 mg. per kilo. This value is based on results obtained with thirty mice, divided in three equal groups, which received 6, 8, and 10 mg. of fumigacin, respectively, per animal. The solution used for injection was prepared by dissolving 1 gm. of fumigacin in about 5 cc. of 1 per cent sodium carbonate solution and diluting it to 100 cc. with sterile distilled water.

Fumigacin when administered in proper dosage was found to afford some protection to mice injected intraperitoneally with 10 to 100 times the

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**Table I**

**Bacteriostatic Effect of Fumigacin**

The minimal concentration of fumigacin in mg. per liter necessary to prevent growth in beef heart-0.25 per cent glucose broth for a specified length of time. The controls showed growth in 4 to 7 hours.

<table>
<thead>
<tr>
<th>Strain</th>
<th>4-7 hrs.</th>
<th>24 hrs.</th>
<th>&gt; 120 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em>, Strain 873</td>
<td>1.5</td>
<td>15</td>
<td>80</td>
</tr>
<tr>
<td>&quot; (Heatley strain)</td>
<td>1.5</td>
<td>14</td>
<td>60</td>
</tr>
<tr>
<td>&quot; albus, Strain 61</td>
<td>1</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>&quot; &quot; 671</td>
<td>1</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>&quot; &quot; 806</td>
<td>0.8</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>&quot; citreus, Strain 74</td>
<td>4</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td><em>Micrococcus tetragenus</em>, &quot; 653</td>
<td>2.5</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td><em>Streptococcus haemolyticus</em>, Strain 660</td>
<td>1.5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>&quot; &quot; C203</td>
<td>2</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>&quot; viridans, Strain 355</td>
<td>39</td>
<td>312</td>
<td>625</td>
</tr>
<tr>
<td><em>Bacillus anthracis</em>, Strain 4094</td>
<td>1.5</td>
<td>156</td>
<td>2500</td>
</tr>
<tr>
<td>&quot; subtilis</td>
<td>1.5</td>
<td>156</td>
<td>2500</td>
</tr>
<tr>
<td>*Streptococcus spec. (non-hemolytic)</td>
<td>7</td>
<td>156</td>
<td>625</td>
</tr>
</tbody>
</table>
lethal dose of *Streptococcus haemolyticus* (Strain C203). In these experiments the solution of the compound was injected subcutaneously, in order to avoid direct contact with the infective organisms. For effective treatment relatively large doses have to be employed in the early stages of the infection. Small amounts (0.5 to 2 mg.) given in a single dose merely retard death, while several such doses given twice daily for 2 to 3 days may prevent it. Amounts of 2 to 4 mg. administered once or twice shortly after the infection were found to be most effective, but even with this mode of treatment protection was afforded at best to only 50 per cent of all infected mice.

*Chemical Studies (A. E. O. Menzel and O. Wintersteiner)*

The culture filtrate was acidified to pH 2 with phosphoric acid and extracted three times with ether, the combined extracts equaling the volume of the filtrate. The ether was evaporated to one-tenth of its volume, shaken repeatedly with saturated sodium bicarbonate solution, which removed a biologically inert, dark red pigment, and then exhaustively extracted with 6 per cent sodium carbonate solution. The ether phase yielded on evaporation gliotoxin (see below). The sodium carbonate solution was acidified and distributed several times with benzene.

Fumigacin—The partly crystalline residue from the benzene (7 to 12 mg. per liter of culture filtrate) yielded on repeated recrystallization from methanol pure fumigacin in the form of filamentous needles (about 3 mg. per liter).

Fumigacin melts with some decomposition at 215–220°, depending on the rate of heating. \([\alpha]^{25}_{D} = -132° \pm 2° \text{ (0.41 per cent in chloroform)}\). The ultraviolet absorption curve shows only strong end-absorption below 260 m\(\mu\) with \(E_{1%}^{1%} = 298\) at 234 m\(\mu\).

\[
\begin{align*}
\text{C}_{27}\text{H}_{38}\text{O}_{8} & \quad \text{Calculated. C 69.84, H 7.69} \\
\text{C}_{27}\text{H}_{38}\text{O}_{8} & \quad \text{" 69.55, " 8.06} \\
\text{Found.} & \quad \text{" 69.55, " 7.74} \\
& \quad \text{" 69.55, " 7.90}
\end{align*}
\]

The equivalent weights obtained by titration with standard sodium hydroxide and phenolphthalein on samples weighing 30 to 60 mg. were 514, 560, and 591. Smaller samples (4 to 10 mg.) gave figures of between 400 and 500.

The figures obtained in two acetyl determinations by the method of Elek and Harte (7) were 7.65 and 9.05 per cent. \(\text{C}_{27}\text{H}_{38}\text{O}_{9}\cdot\text{CO} \cdot \text{CH}_{3}\) would require 8.61 per cent.

Fumigacin is practically insoluble in water, sparingly soluble in cold methanol and ethanol, and more readily so in acetone, ethyl acetate, ben-
zene, and ether. It is easily dissolved by chloroform, acetic acid, and dioxane.

Silver Salt—60 mg. of fumigacin dissolved in 5 cc. of alcohol were neutralized with 1.07 cc. of 0.1 N NaOH. To 3 cc. of this solution were added about 10 cc. of 1 per cent aqueous silver nitrate solution. The resulting crystalline precipitate was washed with water and dried in vacuo at 100° for 2 hours.

\[ C_{32}H_{43}O_7Ag \]

Calculated. C 57.90, H 6.53, Ag 16.27

Found. " 57.77, " 6.56, " 16.14

The remainder was recrystallized by precipitation from chloroform solution with ether.

Found, C 57.82, H 6.57, Ag 16.40

Qualitative Reactions—The following reactions were negative: ferric chloride, Legal, fuchsin sulurous acid, Tollens, Molisch, Rosenheim, Hammersten (for cholic acid), Jaffé-Tortelli, digitonin. The Zimmermann reaction with m-dinitrobenzene for ketones was strongly positive. In the Chabrol-Charonnet test for bile acid (phosphoric acid and vanillin) a strong red color was obtained. Likewise, the Liebermann-Burchard test gave an intense blood-red color. Fehling's solution was slowly but perceptibly reduced at 100°.

In connection with the latter test, which is also given by gliotoxin, it was thought advisable to ascertain whether our preparations were entirely free from the latter compound. Qualitative reactions for sulfur (sodium melt-nitroprusside, sodium plumbite) and a quantitative nitrogen determination on 14 mg. of fumigacin showed this to be the case. Furthermore, 4 mg. dissolved in 2 cc. of Nessler's reagent gave a clear solution which only on prolonged standing deposited a white precipitate, while as little as 0.02 mg. of gliotoxin caused an immediate turbidity.

Action of Alkali—20.84 mg. of fumigacin were dissolved to 2 cc. with 1 N NaOH, and the change in rotation was followed in a 2 dm. tube.

<table>
<thead>
<tr>
<th>Time, min.</th>
<th>5</th>
<th>20</th>
<th>35</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>([a]_D^2) degrees</td>
<td>-27.8</td>
<td>-28.8</td>
<td>-34.6</td>
<td>-44.1</td>
<td>-48.9</td>
<td>-56.6</td>
<td>-69.0</td>
</tr>
</tbody>
</table>

After 4 hours a copious precipitate of well formed plates had settled in the solution. The almost linear rise of the levorotation during the period of observation shows that the reaction had not gone to completion when this point was reached. The crystalline sodium salt thus formed was easily soluble in water, alcohol, and butanol and therefore could be isolated in pure form. Acidification and extraction with ether yielded a resin which could not be crystallized.

A solution of 27.8 mg. of fumigacin in 2 cc. of 0.1 N NaOH was allowed to
stand for 48 hours at room temperature. Titration showed that 0.1095
milliequivalent of alkali had been neutralized. This corresponds to an
equivalent weight of 254 for the reaction product, or to half of the molecular
weight of fumigacin on the basis of the C_{29}H_{38}O_{7} formula.

The methyl ester was prepared with diazomethane in the usual way. It
crystallized from methanol in long, fine needles melting at 260–261°. Its
specific rotation [α]_{D}^{20} was −150° ± 2° (0.57 per cent in chloroform).

\[
\begin{align*}
\text{C}_{13}\text{H}_{14}\text{O}_{7}. & \quad \text{Calculated. C 70.27, H 7.87, OCH₃ 6.06, mol. wt. 512} \\
\text{C}_{13}\text{H}_{14}\text{O}_{7}. & \quad \text{" 70.04, " 8.03, " 6.06, " 514} \\
\text{Found.} & \quad \text{" 69.97, " 8.09, " 6.10, " 501} \\
& \quad \text{" 6.37}
\end{align*}
\]

Oxime—50 mg. of fumigacin were boiled for 3 hours in 3 cc. of a filtered
solution containing 140 mg. of hydroxylamine hydrochloride and 200 mg.
of potassium acetate in 5 cc. of 90 per cent ethanol. The reaction product,
precipitated and washed with water, was repeatedly recrystallized from
70 per cent ethanol. It formed fine needles melting at 204–206°. Found,
N 2.15.

The semicarbazone was prepared in an analogous fashion. After three
recrystallizations from 95 per cent alcohol the melting point remained con-
stant at 225–228°. Found, N 7.22.

Gliotoxin—The ether solution, after extraction of the fumigacin fraction
with sodium carbonate solution, was dried and evaporated. The partly
crystalline residue (about 60 mg. per liter of culture filtrate) was recrystal-
lized repeatedly from ethanol. The melting point of the pure compound
(195° with decomposition, in an open capillary) was not depressed by
admixture of an authentic specimen derived from Gliocladium fimbriatum.²
The specific rotation ([α]_{D}^{20} = −245° in chloroform) and the ultraviolet
absorption spectrum (ε_{max} at 270 mμ, 4500; ε_{min} at 245 mμ, 3500) provided
further proof for the identity with the reference preparation (8).

\[
\begin{align*}
\text{C}_{13}\text{H}_{14}\text{O}_{4}\text{N}_{2}S_{2}. & \quad \text{Calculated. C 47.85, H 4.32, N 8.59, S 19.65} \\
\text{Found.} & \quad \text{" 48.68, " 4.24, " 4.80, " 19.36} \\
& \quad \text{" 48.77, " 4.56}
\end{align*}
\]

The carbon figures obtained in this laboratory on preparations from
Aspergillus fumigatus as well as from Gliocladium fimbriatum were consis-
tently too high and are not in accord with the data of Dutcher (8), which
led this author to revise the C_{14} formula of Weindling. The revised (C_{15})
formula is undoubtedly correct, since it has been confirmed by degradation.²
The possibility that we were dealing in our isolated product with a C_{14}
homologue of gliotoxin appeared remote, since the analytical discrepancy
should then have been noticeable also in the hydrogen and sulfur values;
nevertheless it seemed desirable to establish the number of carbon atoms in our preparation by other means. A methoxyl determination demonstrated the absence of an additional methyl group which might have been attached to one of the two hydroxyl groups in gliotoxin. In order to prove the identity of the underlying heterocyclic ring skeleton in both varieties of gliotoxin, we reduced our compound with hydriodic acid, by which treatment gliotoxin from Gliocladium has been shown to yield a sulfur-free compound $\text{C}_{13}H_{12}O_2N_2$, melting at 122°. The reduction product obtained from our material melted at the same temperature and showed no depression when mixed with the reference preparation. Analysis of the derivative confirmed the $\text{C}_{13}$ formula.

$\text{C}_{13}H_{12}N_2O_2$. Calculated. C 68.40, H 5.26, N 12.28

Found. C 68.65, H 5.64, N 12.65

The microanalyses reported in this paper were carried out by Mr. J. F. Alicino.

SUMMARY

Aspergillus fumigatus when grown on the Czapek-Dox medium produces simultaneously two antibiotically active agents, gliotoxin and fumigacin. The former substance accounts for the greater part of the antibiotic activity. The production of the two compounds under various conditions has been studied. It has been demonstrated that the crystalline material formerly described as fumigacin (1) is a mixture of fumigacin and gliotoxin. Pure fumigacin has been prepared and characterized in regard to its chemical and bacteriological properties. It appears to be identical with the helvolic acid recently isolated by Chain et al. (3) from the same source.

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