XCI. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

LVII. FUMIGATIN (3-HYDROXY-4-METHOXY-2:5-TOLUQUINONE) AND SPINULOSIN (3:6-DIHYDROXY-4-METHOXY-2:5-TOLUQUINONE), METABOLIC PRODUCTS RESPECTIVELY OF *ASPERGILLUS FUMIGATUS* FRESENIUS AND *PENICILLIUM SPINULOSUM* THOM

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DURING the routine examination of a number of cultures of fungi isolated by Mr L. D. Galloway [1936] from Indian soil, our colleague, Mr G. Smith noticed that the metabolism solution of one of them—an indubitable strain of *Aspergillus fumigatus* Fresenius—changed from yellowish brown to a strong purple colour on making alkaline.

We have investigated the reason for this unusual colour change and have shown that it is due to the presence of a hitherto undescribed mould metabolic product which we have called *fumigatin*.

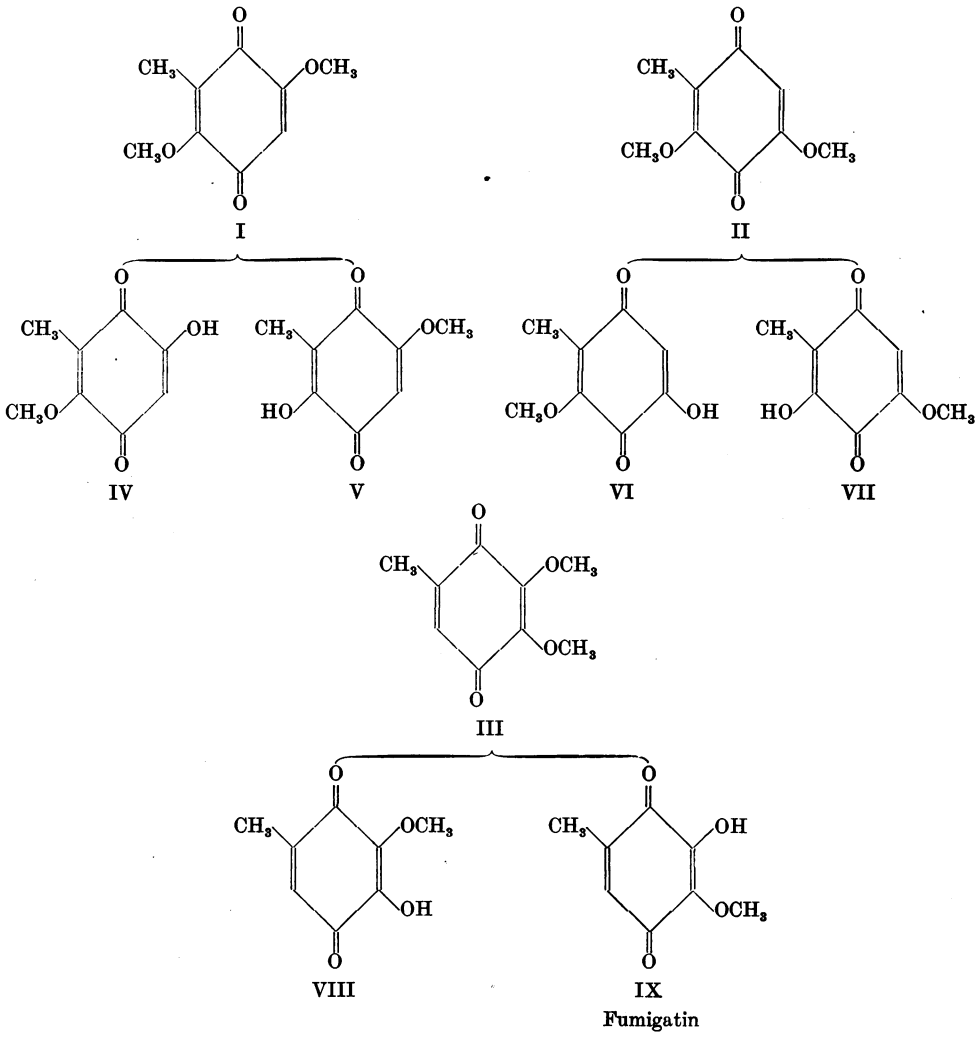
Fumigatin, $C_8H_8O_4$, was isolated as maroon-coloured needles, M.P. 116°. It contains one methoxy group (Zeisel), one methyl group (Kuhn-Roth) and one hydroxy group since it forms a monoacetate and a monomethyl ether. It is without question a monohydroxymonomethoxytoluquinone and the synthesis of fumigatin methyl ether proved conclusively that fumigatin itself is a *p*-quinone and not an *o*-quinone.

There are three possible dimethoxy-2:5-toluquinones, I, II and III, each of which has recently been synthesized here [Anslow *et al.* 1938]. I may arise by methylation of either of the monohydroxymonomethoxy-2:5-toluquinones IV or V, II from VI or VII and III from VIII or IX.

Anslow *et al.* [1938] showed that I melts at 104–105°, II at 125° and III at 59°. Since fumigatin methyl ether melts at 59°, and since its M.P. is not depressed on mixing with III, it follows that fumigatin methyl ether must be III and hence that fumigatin itself must be either VIII or IX.

The ethyl ether of VIII was synthesized by the following series of reactions. Vanillin was reduced to creosol (4-hydroxy-3-methoxytoluene) which was nitrated to 5-nitrocreosol by the method of Oberlin [1925]. This was ethylated to give *5-nitro-3-methoxy-4-ethoxytoluene* which on reduction yielded *5-amino-3-methoxy-4-ethoxytoluene*. On oxidizing this substance *3-methoxy-4-ethoxy-2:5-toluquinone*, i.e. the ethyl ether of VIII was obtained. It melted at 50° and on reduction with sodium hydrosulphite gave the corresponding crystalline quinol *3-methoxy-4-ethoxytoluquinol*, M.P. 64°. On the other hand fumigatin gave an

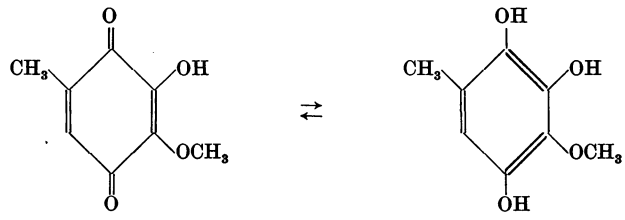
ethyl ether which, on reduction with sodium hydrosulphite, gave the corresponding crystalline quinol, M.P. 55–56° alone, depressed to 46–47° in admixture with 3-methoxy-4-ethoxytoluquinol. Hence fumigatin cannot be VIII and must



be IX, i.e. *3-hydroxy-4-methoxy-2:5-toluquinone*. The quinol, M.P. 55–56°, derived by reduction of the ethyl ether of fumigatin is therefore *4-methoxy-3-ethoxytoluquinol*.

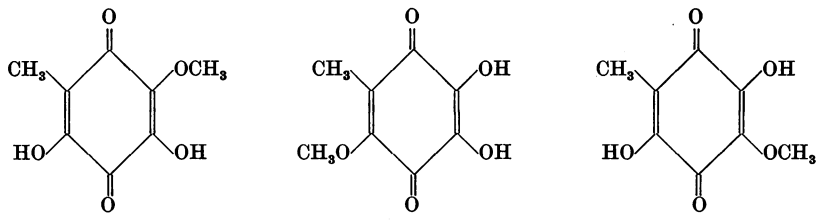
The probable significance of fumigatin in the metabolic processes of the particular strain of *A. fumigatus* used in this investigation is indicated by the fact that we have also isolated from the same freshly separated metabolism solution not only fumigatin, but also its reduction product, the quinol *3-hydroxy-4-methoxytoluquinol* (X). Since these two substances have been shown to be

readily interconvertible, and since they are both undoubted metabolic products, it is difficult to resist the conclusion that they function as an oxidation-reduction system in the life processes of the mould.



Fumigatin X

Birkinshaw & Raistrick [1931] reported the isolation from cultures of strains in the *Penicillium spinulosum* Thom series of a new mould metabolic product which was unnamed at that time, but for which the name *spinulosin* is now proposed. Spinulosin was shown to be a dihydroxymonomethoxytoluquinone and, assuming it to be a *p*-toluquinone, it followed that it must have one of the three formulae XI, XII or XIII.



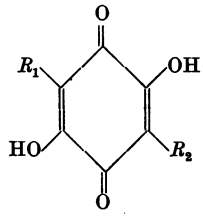
XI XII XIII Spinulosin

We have now shown that fumigatin may be readily converted *in vitro* into spinulosin as follows. Fumigatin was submitted to the Thiele-Winter [1900] reaction when a colourless tetraacetate was obtained identical with the tetraacetate obtained by simultaneous reduction and acetylation of spinulosin. The tetraacetate from fumigatin was hydrolysed to yield the corresponding tetrahydroxymethoxytoluene, which was oxidized, by passing air through its alkaline solution, to give a good yield of spinulosin.

In the Thiele-Winter reaction, which many substituted benzoquinones undergo, a nuclear —CH group, together with both —CO groups and any —C(OH) groups, are acetylated to —C.O.OC.CH_3. Hence, since it is now known that spinulosin is a *p*-quinone, it follows that spinulosin must be a hydroxyfumigatin, the new hydroxyl group occupying the only available position 6; i.e. spinulosin is *3:6-dihydroxy-4-methoxy-2:5-toluquinone* (XIII). The same conclusion was reached by Aulin & Erdtman [1937] by comparing the colour of an alkaline solution of spinulosin with that of certain synthetic dihydroxy-2:5-toluquinones.

The structure assigned to spinulosin brings it into close relationship with certain other naturally occurring derivatives of 2:5-dihydroxy-1:4-benzoquinone,

the constitutions of which have recently been settled. Thus, taking structure XIV as the general formula for these compounds,



XIV

R_1=methyl and R_2=methoxy in spinulosin. In embelic acid (embelin) the active principle of the berries of *Embelia ribes*, Hasan & Stedman [1931] showed that R_1=H and R_2=*n*-lauryl. Kögl and his co-workers have shown that in polyporic acid, isolated from the agaric *Polyporus nidulans* Fr., $R_1=R_2$=phenyl [1926], and in atromentin, which occurs in the agaric *Paxillus atromentosus* Batch, $R_1=R_2$=*p*-hydroxyphenyl [1928].

Experimental

Culture

The culture used throughout the work was, morphologically, undoubtedly a strain of *Aspergillus fumigatus* Fresenius. It was received in January 1936 from Mr L. D. Galloway, who isolated it from Indian soil [Galloway, 1936]. It bears Mr Galloway's catalogue No. CH 3 and the L.S.H.T.M. Cat. No. A 46. No fumigatin could be detected among the metabolic products of five other strains of *A. fumigatus*, i.e. L.S.H.T.M. Cat. Nos. 47, 88, Ac 15, Ac 70 and Ac 71.

Cultural conditions

The culture medium used throughout was a Raulin-Thom solution of the following composition: glucose, 75 g.; tartaric acid, 4·0 g.; ammonium tartrate, 4·0 g.; $(NH_4)_2HPO_4$, 0·6 g.; K_2CO_3, 0·6 g.; $MgCO_3$, 0·4 g.; $(NH_4)_2SO_4$, 0·25 g.; $ZnSO_4$, $7H_2O$, 0·07 g.; $FeSO_4$, $7H_2O$, 0·07 g.; distilled water to 1500 ml. This medium was distributed in 350 ml. amounts in batches of 100 1 l. conical flasks, sterilized, sown with a spore suspension of *A. fumigatus* A 46, and incubated at 24°.

After 31–37 days' incubation the metabolism solution, *p*H 3·3 and still containing 0·5–1 % of sugar, was separated by filtration from the mycelium, which was greyish white in colour, with occasional grey-green sporing patches and with a pale pink to purple reverse. The metabolism solution was yellowish brown in colour.

Fumigatin is present in the metabolism solution partly in the oxidized (quinone) form and partly in the reduced (quinol) form. This is evident since both forms were in fact isolated from the same metabolism solution. It is also indicated by the fact that the freshly separated metabolism solution, when strongly acidified and treated with KI, liberates I equivalent to 4–5 ml. *N*/100 I per 100 ml., while after vigorous aeration it liberates I equivalent to 8–10 ml. *N*/100 I per 100 ml. Since fumigatin in the oxidized (quinone) form is stable in air whilst its reduced (quinol) form is very unstable, it was in general isolated in the quinone form.

Isolation and purification of fumigatin

The filtered metabolism solution was vigorously aerated for 1 hr. by which time the iodine titration had reached a constant value. It was acidified with concentrated HCl (30 ml. per 7 l. of medium corresponding to 20 flasks) and each batch of 7 l. was extracted 8 times with 2 l. $CHCl_3$. This laborious procedure was unfortunately necessary since evaporation of the metabolism solution, even *in vacuo* at 40–45°, caused decomposition of the fumigatin. The $CHCl_3$ extracts were evaporated *in vacuo* (bath temperature not >45°) and the concentrated extracts were allowed to evaporate to dryness in air in open dishes, when reddish brown, crystalline, but somewhat sticky, residues were obtained. The average yield of crude fumigatin so obtained was about 5 g. per 100 flasks. The crude material was dried *in vacuo* over conc. H_2SO_4 and was extracted in a Soxhlet apparatus with light petroleum (B.P. 40–50°) when it was obtained as a maroon-coloured crystalline dry powder (yield about 2 g. of almost pure material per 100 flasks). This was further purified by recrystallization from boiling light petroleum (B.P. 40–50°) in which it is only sparingly soluble (0·17 g. in 200 ml. boiling light petroleum). It separated as maroon-coloured needles, M.P. 116°. For analysis a sample was sublimed in a high vacuum. No decomposition occurred and the M.P. was unchanged.

Isolation of reduced fumigatin (dihydrofumigatin) (3-hydroxy-4-methoxytoluquinol) (X)

The metabolism solution from 20 flasks (residual glucose = 0·66 %) was strained through muslin, acidified with conc. HCl (30 ml.) and extracted four times with 2 l. of ether. The ethereal extracts were evaporated and a portion of the concentrated extract was dried *in vacuo* in a sublimation tube and sublimed twice in a high vacuum. All these operations were performed quickly and as far as possible in the absence of air. The sublimate consisted of a mixture of reddish brown crystals (fumigatin) and a colourless semi-solid material. It was dissolved in ether, and washed with freshly prepared aqueous $NaHCO_3$ which removed fumigatin. The ethereal solution was evaporated to dryness and resublimed. The sublimate was washed with a little CCl_4 which removed traces of impurity and resublimed giving almost colourless needles, M.P. 99–101°. (Found: C, 56·93; H, 6·16 %. $C_8H_{10}O_4$ requires C, 56·45; H, 5·93 %.) The M.P. of a mixture of this substance and a freshly made specimen of dihydrofumigatin (M.P. 100–101°) prepared as described on p. 693 was 100–101°. The two substances also gave the same colour reactions.

General properties of fumigatin

Fumigatin crystallizes in maroon-coloured needles, M.P. 116°, and has the empirical formula $C_8H_8O_4$. (Found on two different samples: C, 57·34, 57·29; H, 4·72, 4·65; OCH_3, 18·5, 18·4 %; N, nil. Mol. wt. cryoscopic in dioxane (Dr A. E. Oxford), 161. $C_8H_8O_4$ requires C, 57·12; H, 4·80; 1 OCH_3, 18·5 %. Mol. wt. 168.) All micro-analyses were carried out by Dr G. Weiler, Oxford.

In a Zerewitinoff determination (Roth), fumigatin afforded 1·2 active H atoms in pyridine at 20° and 1·4 at 95°. On oxidation with chromic acid (Kuhn-Roth method) evidence of one side-chain methyl group was obtained. (Found: 98·8 and 99·9 % of 1 $C_2H_4O_2$.)

Fumigatin readily sublimes, without decomposition, in a high vacuum. It is readily soluble in acetone, ether, chloroform, benzene, ethyl acetate and alcohol, fairly soluble in water and slightly soluble in light petroleum.

It gives the following reactions:

(*a*) A solution in absolute alcohol gives with one drop of *N*/2 $FeCl_3$ an intense purple-black colour which changes to light brown on the addition of water, the latter colour being similar to that given on the addition of $FeCl_3$ to an aqueous solution of fumigatin.

(*b*) With *N* NaOH it gives a colour indistinguishable from that of dilute $KMnO_4$. This colour changes to brown on standing overnight.

(*c*) With aqueous $NaHCO_3$, in which it is immediately soluble, it gives a colour indistinguishable from that of dilute $KMnO_4$. This colour is unchanged overnight.

(*d*) With cold conc. H_2SO_4 it immediately gives a brown colour changing to cherry red after 2 min., and becoming an intense permanganate colour after about half an hour.

(*e*) It dissolves readily in aqueous $Na_2S_2O_4$ to give an almost colourless solution from which dihydrofumigatin may be readily extracted by ether (see later).

(*f*) It liberates I from an acidified solution of KI.

(*g*) It does not react with *o*-phenylenediamine in ether solution standing over anhydrous Na_2SO_4, and can be recovered unchanged. Hence it is probably not an *o*-quinone.

Derivatives of fumigatin

(*a*) *Action of diazomethane.* Fumigatin (0·2 g.), dissolved in ether, was treated with ethereal diazomethane. There was immediate and vigorous evolution of nitrogen and the brownish red solution lightened in colour to orange. The yellow crystals (0·15 g.) separating on removal of most of the solvent were recrystallized from ether and were obtained as lemon-yellow needles, M.P. 93°. (Found: C, 53·46, 53·70; H, 5·15, 5·05; N, 12·6, 12·5; OCH_3, 27·5, 28·0 %. $C_{10}H_{12}O_4N_2$ requires C, 53·56; H, 5·40; N, 12·5; $2OCH_3$, 27·7 %.) This substance has obviously arisen from fumigatin by the methylation of one OH group and the addition of the elements of diazomethane CH_2N_2, with the probable formation of a pyrazole complex—a reaction of frequent occurrence with certain types of quinones.

(*b*) *Fumigatin monomethyl ether* (*3:4-dimethoxy-2:5-toluquinone*) (*III*). Fumigatin (0·2 g.) was dissolved in acetone (5·0 ml.) and methyl sulphate (1·0 ml.) was added. The mixture was boiled for 2½ hr., during which time K_2CO_3 (1 g.) was added in four or five lots. The mixture was cooled, diluted with ether and the K_2SO_4 and excess K_2CO_3 removed by filtration. The solvents were evaporated, leaving a dark-coloured oil which was extracted with boiling light petroleum, B.P. 40–50°. The light petroleum was removed and the residue distilled in a high vacuum giving a reddish brown oil which crystallized later. This separated from light petroleum in long red needles, M.P. 59°. The yield was small. The M.P. was not depressed on mixing with a synthetic specimen of 3:4-dimethoxy-2:5-toluquinone, M.P. 59° [Anslow *et al.* 1938]. (Found: C, 59·29; H, 5·58; OCH_3, 33·2 %. $C_9H_{10}O_4$, i.e. the monomethyl ether of $C_8H_8O_4$, requires C, 59·30; H, 5·53; 2 OCH_3, 34·1 %.)

(*c*) *Fumigatin monoacetate* (*3-acetoxy-4-methoxy-2:5-toluquinone*). Fumigatin (0·2 g.) was dissolved in acetic anhydride (2 ml.) by gentle warming. The solution was cooled and 2 drops conc. H_2SO_4 were added. The mixture was heated but not boiled for a few seconds, when the colour changed from blood red to brownish yellow. The crude acetate (0·14 g.) separating on addition of ice and water was recrystallized from light petroleum (B.P. 40–50°) as canary yellow

rosettes of needles, M.P. 95–96°. A further 0·14 g. was obtained from the filtrate by extraction with ether. (Found: C, 57·42, 57·36; H, 4·96, 4·83; OCH_3, 14·2, 14·5 %. $C_{10}H_{10}O_5$, i.e. the monoacetate of $C_8H_8O_4$, requires C, 57·12; H, 4·80; 1 OCH_3, 14·8 %.)

(*d*) *Dihydrofumigatin* (*3-hydroxy-4-methoxytoluquinol*). Fumigatin (0·2 g.) was shaken for a few moments with $Na_2S_2O_4$ (4 g.) in water (20 ml.) when an almost colourless solution was obtained. This was extracted 4 times with an equal volume of ether and the ether removed, leaving a syrupy residue (0·2 g.), which was dried in a high vacuum at 70°, when it crystallized. The bath temperature was then raised to 90–100°, when the quinol sublimed in colourless micro-crystals, M.P. 100–101°. (Found: C, 56·39, 56·28; H, 5·77, 5·73; OCH_3, 18·3, 18·1 %. $C_8H_{10}O_4$ requires C, 56·45; H, 5·93; 1 OCH_3, 18·2 %.)

Dihydrofumigatin is very readily soluble in water giving a colourless solution which quickly becomes pink. The aqueous solution gave the following reactions:

(i) No precipitate and doubtful absorption with bromine water.

(ii) With $NaHCO_3$ a colour indistinguishable from that of $KMnO_4$ solution and from that given by fumigatin itself with $NaHCO_3$.

(iii) With aqueous $FeCl_3$ a yellow changing to a dark brown colour.

When 2*N* NaOH solution is added drop by drop to the quinol in substance or in concentrated solution, a transient bright green colour is formed, quickly becoming olive green with a purple layer on the surface. With excess NaOH an intense purple colour is formed.

A solution of dihydrofumigatin in absolute alcohol gives with *N*/2 $FeCl_3$ first a brownish yellow colour which, on addition of more $FeCl_3$, suddenly becomes an intense purple-black exactly similar to that given by an alcoholic solution of fumigatin.

Attempts to obtain a crystalline acetyl derivative of dihydrofumigatin have been unsuccessful. In different experiments fumigatin was reduced with $Na_2S_2O_4$ and with zinc dust and acetic acid, and the resultant quinol was acetylated in one case with pyridine and acetic anhydride, and in the other with sodium acetate and acetic anhydride. In both cases there was obtained a very viscous colourless oil which resisted all attempts to crystallize it. Further, a quantitative acetylation of pure dihydrofumigatin by the Peterson-West [1927] method (acetic anhydride in pyridine), while proving conclusively the presence of three acetylatable OH groups (found: 43·4 % $CH_3.CO$; $C_{14}H_{16}O_7$, i.e. the triacetate of $C_8H_{10}O_4$, requires 43·6 %) resulted in a triacetate which proved to be a viscous, colourless, uncrystallizable oil.

(*e*) *Fumigatin monoethyl ether* (*4-methoxy-3-ethoxy-2:5-toluquinone*). Fumigatin (0·168 g. 1/1000 g.-mol.) was dissolved in absolute alcohol (2 ml.) and to the brownish red solution were added 10 ml. of *N*/10 ethyl alcoholic NaOH. The colour changed to an intense purple. 10 ml. of *N*/10 ethyl alcoholic $AgNO_3$ were now added and the Ag salt of fumigatin was precipitated as a purple-black solid, which was separated, washed with alcohol and then with ether. While still damp with ether 1 ml. of freshly distilled ethyl iodide was added together with 10 ml. of ether. An immediate reaction occurred at room temperature, and after 1 hr. the mixture was filtered. The ethereal filtrate was shaken with aqueous $NaHCO_3$ to remove a little unetherified fumigatin, dried and evaporated. The orange red viscous residue, consisting of crude fumigatin ethyl ether, was sublimed in a high vacuum and gave an orange-coloured oil which partly crystallized at 0°. However, because of the small amount available, and because of its unsatisfactory diagnostic value for comparison with synthetic material, it was converted into the corresponding quinol.

(*f*) *Dihydrofumigatin ethyl ether* (*4-methoxy-3-ethoxytoluquinol*). An ethereal solution of sublimed fumigatin ethyl ether, reddish brown in colour, was shaken with aqueous $Na_2S_2O_4$ when the ether layer quickly became colourless. It was separated and the aqueous layer was thoroughly extracted with ether. The combined ether extracts were dried, the solvent removed and the residue sublimed in a high vacuum. On resublimation it was obtained as colourless plates, M.P. 55–56°. A mixture with synthetic 3-methoxy-4-ethoxytoluquinol (M.P. 64°, see below) melted at 46–47°. (Found: C, 60·94, 61·08; H, 7·13, 7·08; total alkoxy calculated as OCH_3, 31·5 %. $C_{10}H_{14}O_4$ requires C, 60·57; H, 7·12; 1 OCH_3+1 OC_2H_5, calculated as % OCH_3, 31·3 %.)

Synthesis of 3-methoxy-4-ethoxy-2:5-toluquinone, i.e. the ethyl ether of VIII and of the corresponding quinol, 3-methoxy-4-ethoxytoluquinol

5-Nitro-3-methoxy-4-ethoxytoluene. Vanillin was reduced to creosol which was nitrated to give 5-nitrocreosol by the method of Oberlin [1925]. A mixture of 5-nitrocreosol (20 g.), diethyl sulphate (20 ml.), K_2CO_3 (50 g.) and toluene (200 ml.) was refluxed for 3 hr. A further 20 ml. of diethyl sulphate were added in portions during the next hour and the heating was continued for 7 hr. in all. Toluene was added to the reaction mixture which was thoroughly washed with 2 % NaOH and dried. The toluene was removed and the residue distilled *in vacuo*. The yellow distillate quickly crystallized, B.P. 165–170°/18 mm. Yield, 13·4 g. A small sample, sublimed in a high vacuum, gave an almost colourless sublimate, M.P. 59°. (Found: C, 57·00, 56·97; H, 5·96, 6·12; N, 6·59, 6·57 %. $C_{10}H_{13}O_4N$ requires C, 56·85; H, 6·21; N, 6·64 %.)

5-Amino-3-methoxy-4-ethoxytoluene. Concentrated HCl (39 ml.) and water (13 ml.) were added slowly to a mixture of granulated tin (18·2 g.), alcohol (26 ml.) and 5-nitro-3-methoxy-4-ethoxytoluene (13 g.). When the evolution of hydrogen had moderated, the mixture was refluxed for 1 hr. The alcohol was removed by distillation *in vacuo* and, after cooling, NaOH (32·5 g.) in water (130 ml.) was added. The amine was extracted with ether and remained after removal of the ether as a pale yellow oil (9·1 g.) which was oxidized to the quinone without further purification.

3-Methoxy-4-ethoxy-2:5-toluquinone. The above amine (9·1 g.) was dissolved in a cold mixture of conc. H_2SO_4 (35 ml.) and water (110 ml.), placed in a freezing mixture and treated with $Na_2Cr_2O_7$ (5·5 g.) in water (33 ml.) slowly with constant stirring. The following day a further 11 g. of $Na_2Cr_2O_7$ in 66 ml. of water were added under the same conditions. After 3 hr. at room temperature the mixture was extracted with ether. On removal of the solvent, 2·7 g. of a dark reddish brown oil remained, which crystallized later at 0°. A portion was sublimed in a high vacuum, dissolved in ether, and the ethereal solution extracted with aqueous $NaHCO_3$ to remove a little impurity, which coloured the aqueous layer deep purple. The ether layer was dried, the solvent removed and the residue sublimed in a high vacuum in long orange-red needles, M.P. 50°. (Found: C, 61·50, 61·30; H, 6·27, 6·30; total alkoxy calculated as OCH_3, 32·4, 32·0 %. $C_{10}H_{12}O_4$ requires C, 61·19; H, 6·17; 1 OCH_3, +1 OC_2H_5, calculated as % OCH_3, 31·6 %.)

3-Methoxy-4-ethoxytoluquinol. 0·2 g. of the above sublimed quinone was dissolved in ether and the ether solution shaken with aqueous $Na_2S_2O_4$, when it rapidly became colourless. It was separated and the aqueous layer was thoroughly extracted with ether. The combined ether extracts were dried, and the solvent removed leaving an oil which later crystallized. The crystals were purified by sublimation in a high vacuum to give colourless prisms, M.P. 64°. Yield almost

quantitative. (Found: C, 60·54, 60·44; H, 6·97, 7·05; total alkoxy calculated as OCH_3, 32·2, 31·8 %. $C_{10}H_{14}O_4$ requires C, 60·57; H, 7·12; 1 OCH_3+1 OC_2H_5, calculated as % OCH_3, 31·3 %.)

Thiele-Winter [1900] reaction on fumigatin. Preparation of 2:3:5:6-tetraacetoxy-4-methoxytoluene

Fumigatin (0·5 g.) was dissolved, at room temperature, in 7·5 ml. of a mixture of acetic anhydride (30 ml.) and conc. H_2SO_4 (1 ml.). The brownish red solution which quickly changed to light brown in colour, after 4 days at room temperature, was poured into ice and water. The crude acetyl compound (0·84 g.) was separated, dried and recrystallized from absolute alcohol, with a little charcoal, in colourless crystals separating in a manner reminiscent of ammonium chloride. (Found: C, 54·44, 54·39; H, 5·25, 5·23; OCH_3, 9·5, 9·7 %. $C_{16}H_{18}O_9$ requires C, 54·23; H, 5·12; OCH_3, 8·8 %.)

2:3:5:6-Tetraacetoxy-4-methoxytoluene melts at 192–192·5°. A freshly isolated specimen of the tetraacetate, prepared by simultaneous reduction and acetylation of spinulosin from *P. spinulosum* [Birkinshaw & Raistrick, 1931] also melted at 192–192·5°. A mixture of the two substances melted at the same temperature.

Conversion of fumigatin into spinulosin (i.e. 3:6-dihydroxy-4-methoxy-2:5-toluquinone (XIII)

The tetraacetyl derivative (0·8 g.), prepared from fumigatin as described in the previous section, was hydrolysed by boiling, in an atmosphere of N_2, for ¾ hr., with 10 ml. of a mixture of methyl alcohol (30 ml.) and conc. H_2SO_4 (1 ml.); the solvent was then evaporated *in vacuo* and replaced by water. The aqueous solution was thoroughly extracted with ether, the ethereal solution dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. The brownish residue, consisting of crude 2:3:5:6-tetrahydroxy-4-methoxytoluene, was dissolved in water (25 ml.) to give a brownish solution. 2*N* NaOH (2·2 ml.) was added to give a definite alkaline reaction and air was rapidly bubbled through. The solution quickly became intensely purple. When the colour appeared to have reached a maximum, a slight excess of 2*N* HCl was added when spinulosin crystallized as a purple-black microcrystalline powder which was separated and dried (0·19 g.). A further 0·05 g. was isolated by ether extraction of the mother liquors. It was purified for analysis by crystallization from toluene and sublimation in a high vacuum. (Found: C, 52·16, 52·39; H, 4·45, 4·39; OCH_3, 17·6, 17·9 %. $C_8H_8O_5$ requires C, 52·17; H, 4·38; OCH_3, 16·9 %.)

This sublimed sample melted at 201°. A freshly sublimed sample of spinulosin isolated from cultures of *P. spinulosum* also melted at 201°. A mixture of the two substances melted at the same temperature.

Each of these specimens gave a bluish purple colour with *N* NaOH, the colour being bluer in shade than $KMnO_4$ solution, a pure and intense blue colour with conc. H_2SO_4 and an intense rich brown colour in alcoholic solution with $FeCl_3$. The colours given by the natural and synthetic specimens of spinulosin in each of the above colour reactions were indistinguishable from each other.

Summary

A hitherto undescribed mould metabolic product *fumigatin*, $C_8H_8O_4$, and its reduction product, $C_8H_{10}O_4$, have been isolated from cultures of *Aspergillus fumigatus* Fresenius grown on Raulin-Thom medium. Fumigatin has been shown to be *3-hydroxy-4-methoxy-2:5-toluquinone* and its reduction product to be

3-hydroxy-4-methoxytoluquinol. It is suggested that these two substances function as an oxidation-reduction system in the vital processes of this mould.

Fumigatin has been converted, *in vitro*, into *spinulosin*, a metabolic product of *Penicillium spinulosum* Thom, described by Birkinshaw & Raistrick [1931]. Spinulosin has been shown to be *6-hydroxyfumigatin*, i.e. *3:6-dihydroxy-4-methoxy-2:5-toluquinone.*

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