CCXCII. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS

LIX. SPINULOSIN (3:6-DIHYDROXY-4-METHOXY-2:5-TOLUQUINONE) A METABOLIC PRODUCT OF A STRAIN OF *ASPERGILLUS FUMIGATUS* FRESENIUS

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ANSLOW & RAISTRICK [1938, 1] reported the isolation, from cultures of a strain of *Aspergillus fumigatus* Fresenius grown on Raulin-Thom medium at 24°, of fumigatin, which they showed to be 3-hydroxy-4-methoxy-2:5-toluquinone. We have now shown that a different strain of *A. fumigatus*, when grown under the same cultural conditions, produces 6-hydroxyfumigatin, i.e. spinulosin (3:6-dihydroxy-4-methoxy-2:5-toluquinone). Spinulosin was first reported by Birkinshaw & Raistrick [1931] as a metabolic product of *Penicillium spinulosum* Thom and its constitution was settled by synthesis by Anslow & Raistrick [1938, 2].

The formation of spinulosin by *A. fumigatus* and *P. spinulosum* adds one more to the small but growing number of examples of species of moulds in different genera which give rise to the same metabolic product. The formation of fumigatin by one strain and of spinulosin by a different strain of the same species of mould is of interest mycologically since, if, as we believe, fumigatin and spinulosin function as oxidation-reduction systems in the vital processes of the moulds which produce them, then their formation points to quite different "oxidation levels" in two authentic strains of the same species of mould. It is of interest to note in this connexion that Anslow & Raistrick [1938, 1] found that only one of six strains of *A. fumigatus* examined gave fumigatin and none produced spinulosin.

EXPERIMENTAL

Culture

The culture used was, morphologically, undoubtedly a strain of *Aspergillus fumigatus* Fresenius. It was isolated by Mr G. Smith in October 1936 from dried Kentish hops, and bears the L.S.H.T.M. Catalogue number A 49. Thom & Church [1926], in their diagnosis of *A. fumigatus*, give "Colonies on Czapek's solution agar in some strains strictly velvety, in others with varying amounts of tufted aerial mycelium up to felted floccose forms." It is interesting to note that colonies of *A. fumigatus* A 49 are almost strictly velvety, sporing readily, whilst *A. fumigatus* A 46, the strain used by Anslow & Raistrick [1938, 1], for the production of fumigatin, gives colonies which are densely floccose, sporing very tardily. The two strains thus represent almost the extreme morphological range of this species.

Cultural conditions

The culture medium used was a Raulin-Thom solution of the following composition: glucose, 75 g.; tartaric acid, 4.0 g.; ammonium tartrate, 4.0 g.; $(\text{NH}_4)_2\text{HPO}_4$, 0.6 g.; K_2CO_3 , 0.6 g.; MgCO_3 , 0.4 g.; $(\text{NH}_4)_2\text{SO}_4$, 0.25 g.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.07 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.07 g.; distilled water to 1500 ml. This medium was distributed in 350 ml. amounts in each of 100 11. conical flasks, sterilized, sown with a spore suspension of *A. fumigatus*, A. 49, and incubated in the dark at 24°.

At the end of the incubation period (25–26 days) the flasks were uniform in appearance, having a crumpled grey mycelium with dark green sporing patches and a grey reverse. The metabolism solution, which was dark purple-red in colour, contained only 0.04% of residual glucose (by polarimeter) and its pH was 5.86. The purple colour was discharged on acidification and became yellow. The solution rapidly reduced permanganate and gave a brown colour with ferric chloride. 50 ml. liberated iodine, from acidified KI solution, equivalent to 4.87 ml. $\text{N}/100 \text{ Na}_2\text{S}_2\text{O}_3$.

Isolation and purification of spinulosin

The filtered metabolism solution from 90 flasks was acidified by the addition of conc. HCl (20 ml./l.) and was then extracted twice, in 2 l. lots, with an equal volume of ether, the ether used for the second extraction of one lot of metabolism solution being used again for the first extraction of another lot. The ethereal extracts were washed with a little water, dried over anhydrous MgSO_4 and evaporated to about 1 l. This ethereal solution was now extracted 4 times with a total volume of 200 ml. of a buffer solution (pH 7.0) made by mixing 50 ml. $\text{M KH}_2\text{PO}_4$ with 29.6 ml. N NaOH and diluting with water to 100 ml. The extracted ethereal solution was redried and evaporated to dryness, giving 1.7 g. of a gummy residue. No evidence of the presence in this fraction of dihydro-spinulosin could be obtained, and nothing crystalline could be isolated except 0.15 g. of an unidentified yellow solid, m.p. 185–190°. The combined buffer solution extracts, which were deep purple in colour, were acidified with conc. HCl and extracted with ether; the ethereal extract was dried and evaporated, giving 2.3 g. of a crystalline, slightly sticky, purple-black residue of crude spinulosin. This was purified by crystallization from toluene, followed by sublimation in a high vacuum and finally by recrystallization from toluene. The final product (0.93 g.) was obtained as lustrous purple-black plates and was identified as spinulosin by its m.p. 200°, alone or in admixture with an authentic specimen, by its colour reactions (a deep pure blue with cold conc. H_2SO_4 , a bluish-purple with 2N NaOH, and a very dark rich brown with FeCl_3 in alcohol), and by analysis. (Found (Weiler): C, 52.32, 52.39; H, 4.34, 4.40; O.CH₃, 16.9, 16.85%. Calc. for $\text{C}_8\text{H}_8\text{O}_5$: C, 52.17; H, 4.38; 1 O.CH₃, 16.9%.)

SUMMARY

Spinulosin (3:6-dihydroxy-4-methoxy-2:5-toluquinone), previously reported as a metabolic product of *Penicillium spinulosum* Thom, has now been isolated from cultures of a strain of *Aspergillus fumigatus* Fresenius. This finding contrasts with the fact that a different strain of *A. fumigatus* gives fumigatin (3-hydroxy-4-methoxy-2:5-toluquinone).

REFERENCES

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