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p. 1419, line 1 of title *for* $C_{20}H_{20}O_5Cl$
read $C_{20}H_{21}O_5Cl$

p. 1419, line 10 of text *for* $C_{20}H_{20}O_5Cl$
read $C_{20}H_{21}O_5Cl$

p. 1420, line 39 of text *for* $C_{20}H_{20}O_5Cl$
read $C_{20}H_{21}O_5Cl$

168. SCLEROTIORINE, $C_{20}H_{20}O_5Cl$, A CHLORINE-CONTAINING METABOLIC PRODUCT OF *PENICILLIUM SCLEROTIORUM* VAN BEYMA

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ONE of the five strains of *Aspergillus terreus* [Raistrick & Smith, 1936] yields two metabolic products, containing chlorine: geodin, $C_{17}H_{12}O_7Cl_2$, and erdin, $C_{16}H_{10}O_6Cl_2$. These compounds, crystallizing in fine yellow needles and melting with decomposition, are the first recorded instances of chlorinated metabolic products of the lower fungi. More recently, the isolation of griseofulvin, $C_{17}H_{17}O_6Cl$ [Oxford *et al.* 1939] and caldariomycin, $C_5H_8O_2Cl_2$ [Clutterbuck *et al.* 1940], chlorinated products of *Penicillium griseofulvum* and *Caldariomyces fumago*, respectively, has been described.

The subject of the present communication, and to which we propose to give the name *sclerotiorine*, $C_{20}H_{20}O_5Cl$, closely resembles the above in crystalline structure but melts without decomposition or formation of a sublimate at 206–7° [Curtin & Reilly, 1940].

The strain of *P. sclerotiorum* used was obtained from the Centraalbureau voor Schimmelcultures and shows under certain well-defined conditions of temperature, mycelial pigmentation, ranging from yellow through orange to red, which colour is particularly apparent in the actual sclerotia. Sclerotiorine is very slightly soluble in cold dil. Na_2CO_3 and $NaHCO_3$ solutions and is obtained in yield of about 2% of the dry mycelium weight.

EXPERIMENTAL

Culture

The organism was first isolated from the air by Prof. Boedijn in Buitenzorg, Java, in 1935 and sent to the Centraalbureau voor Schimmelcultures in May 1936 to be described by van Beyma [1937].

At the optimum temperature, the mould forms masses of orange-red sclerotia, which obscure the blue-green conidia while the reverse is also an intense orange-red. Thus, at 25° pigmentation begins on malt agar in 3–4 days. If, however, the cultures are grown at room temperature the formation of the conidia is almost complete and the appearance of the sclerotia is greatly delayed. The red colouring matter of the organism is soluble in alcohol and ether, giving an orange-yellow solution.

P. sclerotiorum fits Thom's classification of the Monoverticillata stricta.

Cultural conditions

The culture medium used throughout was the well-known Czapek-Dox solution, containing KCl as the sole source of chlorine (glucose, 50 g.; $NaNO_3$, 2 g.; KH_2PO_4, 1 g.; KCl, 0·5 g.; $MgSO_4$, $7H_2O$, 0·5 g.; $FeSO_4$, $7H_2O$, 0·1 g., and distilled water to 1000 ml.).

This medium was distributed in 100 ml. amounts in a batch of 100 20-oz. flat bottles, plugged with cotton wool, sterilized by steaming on each of three consecutive days and sown with a spore suspension of *P. sclerotiorum*, grown for 10 days on malt-agar. The bottles were incubated on the horizontal at approximately 25° in the dark for 24 days. Growth in light gives much inferior mycelium and pigment production.

Mycelium formation became apparent in 2 days and in 4 days a faint orange tint appeared in the upper side of the mycelial felt while the reverse was a bright yellow. After 10 days, the reverse was intensely orange-red and the surface of the mat was salmon pink, interspersed with small white spots. The mixed contents of three bottles were taken every 3 days and tested for *p*H.

Days of incubation	*p*H
0	4·2
3	3·7
6	3·2
9	3·6
12	5·3
15	5·7
18	6·2
21	6·7
24	7·1

After 24 days, the mycelium was strained off and the light golden-yellow medium discarded. When pressed and dried at 50–60° *in vacuo*, the mycelium from 79 bottles weighed 124 g., which corresponds to a yield of 15·5 g. of dry mycelium per initial litre of substrate.

Preparation of sclerotiorine

The finely ground mycelium was completely extracted with light petroleum (B.P. 60–80°) in a Soxhlet apparatus; 0·85 g. of an orange-coloured precipitate was filtered off from the red extract and mycelium, though still coloured, could not be extracted with alcohol. On removal of the light petroleum by distillation, a further 1·60 g. of crude sclerotiorine separated out. At this stage the product melted at 197–201°.

The deep red residue remaining after the complete removal of the light petroleum did not crystallize, and when dissolved in alcohol and diluted with water it gave a cloudy golden orange solution, from which there was no separation of solid material on long standing.

Pure sclerotiorine. The crude colouring matter was recrystallized several times from absolute alcohol and the final product, very fine hair-like yellow needles, melted sharply at 206–207°.

(Found: C, 64·09; H, 5·43%; Cl, 9·47%; OCH_3, nil. Mol. wt. 364. $C_{20}H_{20}O_5Cl$ requires C, 63·92; H, 5·33; Cl, 9·45%. Mol. wt. 376).

Properties of sclerotiorine

(1) Sclerotiorine is insoluble in water but is soluble in most organic solvents.

(2) It dissolves sparingly in cold NaOH, dil. Na_2CO_3, $NaHCO_3$ and NH_4OH solutions. In the latter, it is slightly more readily soluble. The following reactions are noted with the various solutions.

(*a*) The solution in NaOH, yellowish brown, changes to a murky yellow with acid.

(*b*) In Na_2CO_3 and $NaHCO_3$ solutions, the golden yellow coloration shows little change on acidification.

(*c*) The wine-red solution in NH_4OH acidifies to golden yellow, the change being reversible. Sclerotiorine is therefore an indicator and it must be noted that, at the turning point, the solution takes on a cloudy appearance.

(3) With an alcoholic solution of the pigment, NaOH, NH_4OH and Na_2CO_3 give red colorations, but with $NaHCO_3$ the solution remains yellow with a separation of the compound.

(4) Sclerotiorine in alcoholic solution reduces acidified $KMnO_4$, but is not itself reduced by $Na_2S_2O_4$ solution.

(5) Traces of chloride can be detected on prolonged boiling of the pigment with distilled water. On heating with NaOH and acidifying with HNO_3, a precipitate is obtained on the addition of $AgNO_3$ solution.

(6) On addition of alkali to the orange-yellow alcoholic solution, a red coloration is obtained and this is decolorized by Zn dust and heating.

(7) The alcoholic solution does not give a coloration with alcoholic $FeCl_3$.

(8) The addition of much water to the alcoholic solution precipitates the pigment.

(9) Sclerotiorine does not show oxonium salt formation with anhydrous HCl in absolute ether.

(10) With conc. H_2SO_4 the compound yields a bright red-orange colour, changing to yellow on dilution.

(11) Sclerotiorine sublimes in a high vacuum at 175–180°. The sublimate of microscopic needles melts unchanged at 206–207°.

(12) In alcoholic solution, sclerotiorine does not liberate iodine from acidified KI solution. It is not, therefore, a *p*-quinone.

On dilution of the alcoholic mother liquors of crystallization of sclerotiorine, a red compound, melting at approximately 150°, separates out in yield too small for examination.

The properties of sclerotiorine are being further investigated.

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