CLXXXVIII. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS.

LI. THE METABOLIC PRODUCTS OF *ASPERGILLUS TERREUS* THOM.

PART II. TWO NEW CHLORINE-CONTAINING MOULD METABOLIC PRODUCTS, GEODIN AND ERDIN.

By HAROLD RAISTRICK and GEORGE SMITH.

From the Division of Biochemistry, London School of Hygiene and Tropical Medicine, University of London.

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In a previous communication [Raistrick & Smith, 1935] the metabolism at 24° of five strains of *Aspergillus terreus* Thom on the well-known Czapek-Dox solution, which contains KCl as the sole source of chlorine, was described. It was shown that two of the strains, Ac 100 and No. 45, produced a new water-soluble mould metabolic product, terrein, $C_8H_{10}O_3$. From all five strains the metabolism solutions gave, after the glucose was nearly all consumed, precipitates on addition of HCl, the largest amounts being given by the two strains Ac 100 and No. 45. Ether extraction of the acid precipitate from Ac 100 gave citrinin $C_{13}H_{14}O_5$, the crystalline yellow colouring matter first isolated from *Penicillium citrinum* Thom [Hetherington & Raistrick, 1931], whilst extraction of the precipitate from No. 45 gave a crude crystalline material, M.P. 193–196° decomp., the composition of which forms the subject of the present communication. This material consists essentially of a mixture of two new mould metabolic products containing chlorine, for which the names proposed are *geodin*, $C_{17}H_{12}O_7Cl_2$, M.P. 235° (decomp.), and *erdin*, $C_{16}H_{10}O_7Cl_2$, M.P. 211° (decomp.). The properties and some derivatives of these products are described.

Since it is believed that this is the first recorded instance of one of the lower fungi giving rise to metabolic products containing chlorine, it was necessary to prove that these substances are true metabolic products and not artefacts. The metabolism solution shows very strong reducing properties and, since HCl was used for acidification, it was possible that the chlorinated compounds were formed by addition of HCl to some very reactive substance in solution. Even if it were proved that the same compounds could be obtained by using H_2SO_4 for acidification there would still be a doubt, unless it could be shown that the KCl originally present in the culture medium disappears during the course of metabolism. An experiment was therefore carried out in which estimations of chloride in the metabolism solution were made every few days during the incubation period. It was found that, after an initial lag of a few days during which a good mycelial felt was established, the amount of chloride ion in solution diminished at approximately the same rate as the glucose and, after no further utilization of glucose occurred, the amount of chloride in solution was approximately 6 % of the quantity originally present. At this stage addition of H_2SO_4 to the metabolism solution, while removing practically no chloride from the solution, gave a heavy precipitate, identical in appearance with that obtained by using HCl, and containing almost 50 % of the chlorine originally present (as KCl) in the culture medium. From the acid precipitate thus obtained, pure samples of geodin and erdin were isolated. There can thus be no doubt that these two substances are true metabolic products. A further confirmation is provided by the fact that neutral solutions of the sodium salts of both geodin and erdin give, on addition of $FeCl_3$, heavy grey precipitates turning brown on standing, a characteristic reaction given also by the metabolism solution of the mould.

It obviously became of interest to test whether brominated and iodinated metabolic products corresponding to geodin and erdin could be isolated from metabolism solutions of *A. terreus*, grown on Czapek-Dox media containing equivalent amounts of KBr or KI in place of KCl. Unfortunately, although practically normal growth was obtained on such media, no metabolic products containing bromine or iodine could be isolated.

It is of interest to note the announcement by Nolan [1934; Hardiman *et al.* 1935] of the isolation of two lichen acids containing chlorine, gangaleoidin, $C_{18}H_{14}O_7Cl_2$, from *Lecanora gangaleoides*, and diploicin, $C_{16}H_{10}O_5Cl_4$, from *Buellia canescens*. Although we have as yet no evidence of structural relationship, the close similarity between the empirical formulae of these lichen acids and of the new mould products is suggestive.

Work is at present in progress on the molecular constitution of geodin and erdin and will be reported in a future communication.

EXPERIMENTAL.

Details of the cultural conditions and of the method of isolation of the crude ether-soluble fraction of the acid precipitate have already been given [Raistrick & Smith, 1935, p. 609].

Chlorine metabolism.

100 flasks of Czapek-Dox solution (glucose, 50 g.; $NaNO_3$, 2·0 g.; KH_2PO_4, 1·0 g.; KCl, 0·5 g.; $MgSO_4, 7H_2O$, 0·5 g.; $FeSO_4, 7H_2O$, 0·01 g.; distilled water, 1 litre), 350 ml. in each litre conical flask, were sown with a spore suspension of *A. terreus*, strain No. 45, and incubated at 24°. Single flasks were used at intervals for estimation of residual chloride in solution and of apparent residual glucose by polarimeter. Direct estimation of chloride was rendered impossible by the fact that the metabolism solution, after about 14 days' incubation, rapidly reduces $AgNO_3$. Treatment with lead acetate removes interfering substances, but the precipitate carries down some chloride even although the total chloride in the original culture medium is less than that contained in a saturated solution of $PbCl_2$. It was found, however, that norit charcoal will completely remove all interfering substances and does not adsorb any chloride. The metabolism solution, when the glucose has been reduced to about 1 %, is dark brown in colour, gives a thick brown precipitate when acidified, reduces $AgNO_3$, $KMnO_4$ or iodine rapidly in the cold and gives a heavy grey precipitate on addition of $FeCl_3$. After treatment in the cold with 4–5 % of norit the solution is colourless, gives no precipitate on acidification, does not reduce $AgNO_3$, reduces $KMnO_4$ only slowly and gives no reaction with $FeCl_3$. The minimum amount of norit required is 4 g. per 100 ml. solution until the glucose is reduced to about 0·4 % and thereafter 5 g. per 100 ml. It was shown by tests on Czapek-Dox solution that norit does not adsorb any of the chloride in the medium but that the solution extracts a very small amount of chloride from the norit, equivalent to 0·0016 g. KCl per 5 g. norit. This was, of course, allowed for in all estimations.

The actual method of estimation was to shake 100 ml. of metabolism solution with 5 g. of norit for 10 min. in the cold, filter and collect exactly 85 ml. of clear filtrate, add HNO_3 and standard $AgNO_3$ solution and titrate the excess silver by the Volhard method. The results are shown in Table I.

Table I.

Days' incubation	Apparent residual glucose by polarimeter %	Residual chloride as KCl %
0	5·00	0·0497
9	2·85	0·0420
12	1·77	0·0338
14	1·50	0·0323
17	0·85	0·0161
19	0·23	0·0072
21	0·81	0·0116
24	0·35	0·0057
26*	0·37	0·0034
Acid filtrate		0·0028

* Average of 91 flasks.

The figures take no account of evaporation of the medium during incubation. Actually, at the end of the period of incubation, the volume of filtered metabolism solution from 100 flasks, originally containing 35 litres of medium is, on the average, 31 litres.

There was some irregularity amongst individual flasks, and the figures obtained from analyses of the contents of single flasks do not give smooth curves for consumption of glucose and chloride. They show, however, that after an initial period during which the mycelial felt is being established the chloride disappears at approximately the same relative rate as the glucose.

After 26 days' incubation the contents of the remaining 91 flasks were filtered and the filtrate, 28 litres, divided into two equal portions, one being made acid to Congo red by addition of 75 ml. concentrated HCl, and the other acidified with an equivalent amount (375 ml.) of $2N$ H_2SO_4. Flocculent brown precipitates were formed in both solutions, the two being identical in appearance. The precipitates were filtered off, washed with water until free from acid and chloride and dried *in vacuo*. The two were identical in weight, 15·5 g. Residual chloride was estimated in the filtrate obtained after acidification with H_2SO_4. As seen from Table I it was slightly less than before acidification but the difference is very small compared with the total amount of chlorine in the precipitate. Estimation of chlorine in the crude H_2SO_4 precipitate gave 11·4 %. The chloride originally present in the culture medium is therefore accounted for as follows:

0·0497 % KCl in 15·925 litres medium (half of 91 flasks) = 3·764 g. Cl.
0·0034 % KCl in 14 litres metabolism solution = 0·226 g. Cl = 6 % original.
11·4 % Cl in 15·5 g. acid precipitate = 1·767 g. Cl = 47 % original.
Unaccounted for = 1·771 g. Cl = 47 % original.

The two acid precipitates, obtained by acidification with HCl and H_2SO_4 respectively, were exhaustively extracted with ether, side by side in Soxhlet extractors. The crystalline material collecting in the flasks was filtered off at intervals and the ether mother-liquors were evaporated to obtain further crops until only tarry matter remained. The total amounts of crude crystalline material obtained were, respectively, *ex* HCl precipitate 7·25 g.; *ex* H_2SO_4 precipitate 7·28 g. The two lots were apparently identical and both were shown, by fractional crystallization as described below, to consist essentially of geodin and erdin.

Fractionation of crude crystalline material.

Fractional extraction with ether of the combined acid precipitates from several 100-flask experiments resulted in a partial separation of the two substances. The more soluble fractions melted at about 195° with blackening and vigorous evolution of gas and gave a colourless crystalline sublimate when further heated to 230°. The first crops obtained from the later extracts, representing the less soluble material, melted at about 220° with evolution of gas but did not blacken or give any sublimate on further heating to 250°. All the fractions were pale buff in colour and dissolved readily in dilute NaOH to give bright yellow solutions which could be back-titrated quite sharply with HCl. The various fractions were sorted according to the optical rotations of the neutral solutions. For the fractions of low M.P. $[\alpha]_{5461}$ varied from $+7°$ to $+50°$, whilst fractions which showed no blackening on heating gave $[\alpha]_{5461}$ approximately $+70°$. Calculations from the specific rotations, under these conditions, of geodin $(+72°)$ and erdin (zero) showed that the two substances were present in approximately equal amounts in the crude material. Attempts at further fractionation with ether were unsuccessful, the two substances having almost equal solubilities, and hence the preliminary separation depended on the relative solubilities in presence of the brown tarry material which was always extracted along with the crystalline products. A fairly clean separation was effected by using $CHCl_3$, in which geodin is readily soluble and erdin very sparingly soluble. Each crude fraction was boiled with $CHCl_3$, 10 ml. for each 1 g. of geodin present, the solutions being filtered hot and diluted with 2 vol. of ether. On cooling, geodin separated in good yield in rosettes of fine needles, pale brown in colour, M.P. 225–230° (decomp.). Recrystallization from ethyl acetate gave fine needles, still brownish, M.P. 235° (decomp.), and further recrystallization did not raise the M.P. By sublimation in a high vacuum at 200° the trace of brown colouring matter was eliminated, the sublimate consisting of a micro-crystalline powder of a clear pale yellow colour, M.P. 235° with evolution of gas but without any sublimate.

The residues from the $CHCl_3$ extractions were crystallized several times from ethyl acetate and erdin was thus obtained as pale yellow needles, M.P. 211°, blackening with evolution of gas and giving a sublimate in the form of long colourless needles when heated to 230°.

It was shown, by fractional crystallization of the crude crystalline material from ethyl acetate, that geodin and erdin do not owe their chlorine content to the use of $CHCl_3$ as solvent, but this method is much more tedious than the one involving the use of $CHCl_3$.

PROPERTIES AND ANALYSES OF THE NEW METABOLIC PRODUCTS.

All analyses except OCH_3 estimations were carried out by Schoeller (Berlin). Determinations of mol. wt. by the cryoscopic method were carried out by Dr A. E. Oxford, to whom we tender our best thanks.

Geodin.

Geodin, $C_{17}H_{12}O_7Cl_2$, crystallizes from a mixture of 1 vol. $CHCl_3$ and 2 vol. ether as pale yellow, fine needles, M.P. 235°, with evolution of gas but without blackening or the formation of a sublimate. (Found: (*a*) on sample *ex* HCl precipitate, C, 51·10, 51·01; H, 3·09, 3·00; Cl, 17·61, 17·80; OCH_3, 15·55, 15·87 %. Mol. wt. cryoscopic in dioxan, 380; (*b*) on sample *ex* H_2SO_4 precipitate, C, 51·10; H, 3·00; Cl, 17·46 %. $C_{15}H_6O_5Cl_2\,(OCH_3)_2$ requires C, 51·13; H, 3·03; Cl, 17·77;

$2OCH_3$, 15·55 %. Mol. wt. 399.) Optical rotation: in $CHCl_3$ ($c=0·8$), $[\alpha]^{20°}_{5461}+179°$, $[\alpha]^{20°}_{5790}+149°$.

Geodin is insoluble in water or light petroleum, very sparingly soluble in benzene or ether, somewhat more soluble in alcohol or ethyl acetate and dissolves readily in chloroform, acetone or dioxan. The solution in alcohol gives on addition of $FeCl_3$ solution a dirty green colour changing to brown, whilst a neutral solution of the sodium salt gives a heavy brownish grey precipitate. The substance dissolves readily in $NaHCO_3$ solution.

Titration. If dissolved in a slight excess of *N*/10 NaOH and the solution immediately back-titrated with *N*/10 HCl, geodin behaves as a dibasic acid. Equivalent thus found 198; 199. On long standing, or on heating with excess of alkali, decomposition takes place. The neutral solution of the disodium salt has $[\alpha]^{20°}_{5461}+72·4°$, $[\alpha]^{20°}_{5790}+65·6°$.

Identification of alkoxyl groups. 1 g. of geodin was dealkylated by heating with HI and the gaseous products were passed into freshly distilled dimethylaniline. Colourless plates (1·03 g.) were rapidly deposited and, at the end of the reaction, were filtered off, washed with ether and recrystallized from absolute alcohol. (Found: I, 48·1 %. $C_6H_5.N(CH_3)_3I$ requires I, 48·25 %.) The corresponding compound $C_6H_5.N(CH_3)_2.C_2H_5I$ is not crystalline and requires I, 45·8 %. Hence the alkoxyl groups are methoxyl.

Action of diazomethane on geodin. Geodin (1 g.) was suspended in dry ether and treated with an excess of ethereal diazomethane. There was vigorous evolution of nitrogen and the mixture turned bright pink. At the end of the reaction there was present a considerable amount of red crystalline solid. This was filtered off and washed with ether; wt. 0·93 g., M.P. 151° decomp. A further 0·03 g. was obtained by evaporation of the ethereal mother-liquor. Under the microscope the material appeared as colourless crystals with a small amount of red dye adsorbed rather than as red crystals. By short boiling in MeOH solution the red colour was destroyed and, on cooling, the solution deposited fine colourless needles, M.P. 151° decomp.; insol. in $NaHCO_3$. (Found: C, 50·31, 50·35; H, 3·51, 3·56; N, 6·31, 6·27; Cl, 15·35, 15·64; OCH_3, 20·33, 20·37 %. Mol. wt. cryoscopic in nitrobenzene, 419. $C_{16}H_7O_4N_2Cl_2(OCH)_3$ requires C, 50·10; H, 3·54; N, 6·15; Cl, 15·58; $3OCH_3$, 20·43 %. Mol. wt. 455). Treatment with diazomethane therefore results in the introduction of one new methyl group and the addition of one molecule of CH_2N_2, presumably at a double bond. The substance is optically active, having, in benzene ($c=0·9$) $[\alpha]^{22°}_{5461}-58°$, $[\alpha]^{22°}_{5790}-44°$.

Catalytic reduction; *dihydrogeodin.* Geodin (1 g.), dissolved in cold absolute alcohol (250 ml.) $[\alpha]^{25°}_{5461}+152°$ was shaken with hydrogen in presence of a catalyst prepared from palladium chloride (0·5 g.) and charcoal (1·5 g.). The uptake of gas was very rapid, being complete in 30 sec., and the volume absorbed corresponded with the entry of two hydrogen atoms into the molecule. The catalyst was filtered off and the alcoholic solution, now optically inactive, was evaporated *in vacuo* to 40 ml. On addition of 2 vol. of H_2O dihydrogeodin separated as fine needles of a bright yellow colour, M.P. 216–217° decomp. raised to 229° *ex* ether-light petroleum; yield 0·84 g. (Found: C, 51·05, 50·76; H, 3·72, 3·70; Cl, 17·59, 17·45; OCH_3, 15·46 %. $C_{17}H_{14}O_7Cl_2$ requires C, 50·87; H, 3·52; Cl, 17·68; $2OCH_3$, 15·47 %.) Dihydrogeodin is readily soluble in alcohol, ether, ethyl acetate or acetone and almost insoluble in H_2O, $CHCl_3$, C_6H_6 or light petroleum. Its alcoholic solution gives an olive-green colour with $FeCl_3$.

Acetylation of geodin. Attempts to prepare an acetyl compound of geodin have so far been unsuccessful. Treatment with acetic anhydride and pyridine in the cold led to dark brown tarry products. Heating geodin (1 g.) with a mixture of acetic anhydride (4 ml.) glacial acetic acid (6 ml.) and anhydrous sodium acetate (2 g.) at 140–150° for 20 min. gave a mixture of substances which were apparently acetyl derivatives of breakdown products.

Erdin.

Erdin, $C_{16}H_{10}O_7Cl_2$, was obtained by crystallization from ethyl acetate as fine yellow needles, M.P. 211°, with blackening and vigorous evolution of gas. Frothing increases as the temperature is raised and at 225–230° a sublimate collects in the upper part of the M.P. tube in the form of long colourless needles.

Erdin clings most tenaciously to the solvents used for its purification and these cannot be removed completely even in a high vacuum. Hence the analyses submitted below are not in themselves sufficiently conclusive to establish beyond doubt the empirical formula for erdin. Fortunately the crystalline product obtained by the action of diazomethane on erdin (see below) was obtained free from solvent and the results of its analysis leave no room for doubt that erdin itself has the empirical formula $C_{16}H_{10}O_7Cl_2$.

Thus a sample of erdin recrystallized from ethyl acetate containing a little ethyl alcohol lost no weight on heating in nitrogen at 110°, but apparently contained 0·5 mol. of ethyl alcohol. (Found: C, 50·08, 50·06; H, 3·31, 3·29; Cl, 17·20, 17·30; OCH_3, 11·5, 11·4%. Mol. wt. cryoscopic in dioxan, 297, 292. $C_{15}H_7O_6Cl_2(OCH_3)$, $0{\cdot}5C_2H_5OH$ requires C, 50·00; H, 3·21; Cl, 17·38; OCH_3 (1·5 groups) 11·4%. Mol. wt. if completely dissociated in dioxan, 272. $C_{15}H_7O_6Cl_2(OCH_3)$ requires C, 49·87; H, 2·62; Cl, 18·42; $1OCH_3$, 8·06%. Mol. wt. 385.)

Erdin crystallizes from a mixture of 1 vol. dioxan and 2 vol. H_2O in beautiful yellow rectangular plates containing 1 mol. dioxan and 2 mol. H_2O of crystallization, M.P. 193° decomp. (Found on air-dried sample: C, 47·31, 47·46; H, 4·31, 4·30; Cl, 14·03, 13·80%. $C_{16}H_{10}O_7Cl_2$, $2H_2O$, $C_4H_8O_2$ requires C, 47·14; H, 4·35; Cl, 13·93%.) OCH_3 could not be estimated since dioxan is decomposed by HI to give an indeterminate amount of C_2H_5I. A sample dried to constant weight at 135° lost 23·70% and had M.P. 210–211°. Calculated loss of $1{\cdot}5H_2O + C_4H_8O_2$, 22·6%. Analysis of the dried product showed that 0·5 mol. H_2O was still retained. (Found: C, 49·00, 49·01; H, 2·88, 2·84; Cl, 17·82, 17·84; OCH_3, 7·83, 7·96%. $C_{15}H_7O_6Cl_2(OCH_3)$, $0{\cdot}5\,H_2O$ requires C, 48·73; H, 2·81; Cl, 18·00; $1OCH_3$, 7·87%.) Further heating at 150° in a high vacuum resulted in no further loss. (Found: C, 48·98, 49·13; H, 2·82, 2·71; Cl, 17·93, 17·96%.) At higher temperatures obvious decomposition occurred. The substance could not be sublimed unchanged in the highest vacuum attainable with a mercury diffusion pump.

Titration. Erdin titrates in the same way as geodin, reacting as a dibasic acid and decomposing slowly in presence of excess of alkali; equiv. wt. 192. $C_{16}H_{12}O_7Cl_2$, $0{\cdot}5H_2O$, as a dibasic acid requires 197.

Optical rotation. Both erdin itself and its disodium salt are optically inactive.

The solubilities of erdin are closely similar to those of geodin except that it is only very slightly soluble in $CHCl_3$. It also shows reactions with $FeCl_3$ identical with those given by geodin, giving a dirty green colour turning brown in alcoholic solution, and a heavy grey precipitate as the sodium salt in H_2O. Like geodin also erdin dissolves readily in $NaHCO_3$ solution.

Action of diazomethane on erdin. Erdin (1 g.) was suspended in dry ether and treated with an excess of ethereal diazomethane. The reaction proceeded exactly as with geodin, the product being bright pink and almost insoluble in ether; yield 0·94 g.; M.P. 154°; insol. in $NaHCO_3$. By crystallization from MeOH colourless needles were obtained, M.P. 154° (decomp.), mixed M.P. 142–144° with the corresponding product from geodin (M.P. 151°). (Found: C, 50·15, 50·32; H, 3·62, 3·53; N, 6·21, 6·22; Cl, 15·53, 15·69; OCH_3, 20·45, 20·32 %.. Mol. wt. cryoscopic in nitro-benzene 435. $C_{16}H_7O_4N_2Cl_2(OCH_3)_3$ requires C, 50·10; H, 3·54; N, 6·15; Cl, 15·58; $3OCH_3$, 20·43 %. Mol. wt. 455.) By the action of diazomethane, therefore, two new methoxyl groups have been introduced and one molecule of CH_2N_2 added on, the product having the same empirical formula and containing the same number of methoxyl groups as the corresponding product from geodin. Unlike the latter, however, the product from erdin is optically inactive. This fact, together with the depression in M.P. noted above, leaves no doubt that the substances are not identical.

Catalytic reduction; *dihydroerdin*. Erdin (1 g.) dissolved in absolute alcohol (100 ml.) was shaken with hydrogen in presence of a palladium-charcoal catalyst. Hydrogenation proceeded exactly as in the case of geodin, 2 atoms of hydrogen being taken up in approximately 30 sec. The catalyst was filtered off, the solution evaporated *in vacuo* to 40 ml. and water added till the solution was faintly turbid. The product crystallized in bright yellow needles (0·86 g.), M.P. 240° with evolution of gas and formation of a colourless crystalline sublimate resembling that obtained from erdin itself. For analysis the material was dried at 80° over $CaCl_2$ *in vacuo*, there being no further loss in weight at 110° *in vacuo*. (Found: C, 48·90, 48·92; H, 3·38, 3·27; Cl, 17·80, 17·77 %. $C_{16}H_{12}O_7Cl_2$ requires C, 49·61; H, 3·13; Cl, 18·32 %. $C_{16}H_{12}O_7Cl_2, 0{\cdot}5H_2O$ requires C, 48·48; H, 3·31; Cl, 17·90 %.) Dihydroerdin, like the parent substance, erdin, thus contains 0·5 mol. of water of crystallization which cannot be removed even by intensive drying.

Dihydroerdin, which is optically inactive, is readily soluble in ether, alcohol, ethyl acetate or acetone, and practically insoluble in H_2O, light petroleum, $CHCl_3$; or C_6H_6. In alcoholic solution it gives an olive-green colour with $FeCl_3$. 0·5 g. treated with ethereal diazomethane gave a thick yellow oil which could not be crystallized.

Acetylation. On acetylation of erdin with acetic anhydride, glacial acetic acid and anhydrous sodium acetate, a mixture of substances was obtained which has not yet been satisfactorily fractionated.

EXPERIMENTS ON CULTURE MEDIA CONTAINING BROMIDE AND IODIDE.

Experiments were carried out with a view to finding whether *A. terreus* can utilize bromide and iodide and build up metabolic products similar to geodin and erdin but containing bromine or iodine in place of chlorine.

35 litres of a modified Czapek-Dox solution, containing 28 g. KBr in place of 17·5 g. KCl, were distributed in 100 flasks in the usual way and sown with a spore suspension of *A. terreus* No. 45. Growth was rapid and very similar to that on the normal medium. Attempts to estimate residual bromide were unsuccessful, the method used for estimation of chloride being inapplicable owing to the norit adsorbing an appreciable amount of bromide from solution. After 24 days the residual glucose was 0·37 % (polarimeter), the solution being dark brown and giving a definite precipitate on addition of mineral acid. The contents of all the flasks were therefore filtered and the clear solution acidified by addition of 700 ml. of $2N$ H_2SO_4. The precipitate was filtered off, thoroughly washed and dried *in vacuo*. It was thus obtained as a brown powder, 10·7 g. No halogen could be detected in the dry precipitate by the usual tests. Extraction with ether gave a considerable amount of tar and a small quantity (0·85 g.) of brownish crystalline material, M.P. 200–210° decomp. Tests for the presence of bromine in this material were negative.

In a second experiment the KCl of Czapek-Dox solution was substituted by an equivalent quantity (39 g.) of KI. Growth was rapid and fairly normal, there being somewhat more variation amongst individual flasks than when the normal medium was used. After 24 days the glucose in solution was reduced to 0·31 %, and the contents of all the flasks were then worked up. The dry acid precipitate, 10 g., which was almost black and carbonaceous, contained no iodine and gave only a small amount of tar on extraction with ether.

SUMMARY.

A strain of *Aspergillus terreus* Thom, when grown at 24° on Czapek-Dox solution containing KCl as sole source of chlorine, gives rise to two hitherto undescribed, chlorine-containing metabolic products for which the names *geodin*, $C_{17}H_{12}O_7Cl_2$, and *erdin*, $C_{16}H_{10}O_7Cl_2$, are proposed. Methods of isolation and certain derivatives are described. The substitution of KBr or KI for KCl in the Czapek-Dox medium did not result in the isolation of brominated or iodinated metabolic products, although practically normal growth of the mould was obtained.

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