

3. In the Gouy diffusimeter the nephrotic serum albumins generally diffused more slowly, and the urinary albumins more rapidly, than normal.

4. It has been demonstrated that nephrotic albumins are abnormal. Probably the mean molecular weights of nephrotic serum and urinary albumins are respectively higher and lower than normal.

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Studies in the Biochemistry of Micro-organisms

89. METABOLIC PRODUCTS OF *PENICILLIUM MULTICOLOR* G.-M. AND P. WITH SPECIAL REFERENCE TO SCLEROTIORIN

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During the routine testing of a number of moulds for new metabolic products five strains of *Penicillium multicolor* Grigorieva-Manilova and Poradielova came under observation. Four of these five strains produced in the mycelium a yellow colouring matter which was extractable with light petroleum and which bore a strong resemblance to a chlorine-containing compound obtained from *P. sclerotiorum* van Beyma by Curtin & Reilly (1940) and named by them sclerotiorin(e). Sclerotiorin had already been obtained independently in these laboratories in 1940 by Dr R. F. Hunter from the mycelium of *P. sclerotiorum*. This finding was not recorded in the literature since publication was forestalled by Curtin & Reilly (1940), but there was no doubt from the source of the substance, its composition (particularly its chlorine content) and a com-

parison of properties that Dr Hunter's isolate was identical with sclerotiorin. Since Hunter's preparation was still available it was compared with the substance isolated from the various strains of *P. multicolor* and identity was established.

One of the strains of *P. multicolor* (no. N.R.R.L. 2324, Northern Regional Research Laboratory, Peoria, Illinois) afforded, in addition to sclerotiorin in the mycelium, a culture solution which on acidification to about 0.5N with hydrochloric acid gave a deposit of crystalline needles. This apparently new product, denoted as product I, was somewhat soluble in water but was reprecipitated on acidification. It had m.p. 110.5° and gave in aqueous solution a cherry-red colour with ferric chloride. From the analysis and determination of the equivalent the new product had the probable empirical formula

$C_{13}H_{16}O_6$. Extraction with ether of the filtrate obtained after precipitation of the above product afforded a second new product, product II, as pale yellow leaflets, softening at 128–132° and clearing at 133–134° after preliminary shrinking. In aqueous solution this product also gave a cherry-red colour with ferric chloride. Analysis indicated an empirical formula of $C_{13}H_{18}O_7$. These products were isolated only from the first batch of flasks of strain no. N.R.R.L. 2324. Since further supplies were unobtainable these substances could not be further investigated.

Advantage was taken of the fact that higher yields of sclerotiorin were obtainable from *P. multicolor* than had been recorded from *P. sclerotiorum*, to prepare a quantity of the material from the former species after selecting the highest yielding strain. It was thus possible to characterize the material more completely by the preparation of several crystalline derivatives and by further analyses of sclerotiorin itself and of the derivatives to advance a more firmly based empirical formula. Sclerotiorin was found to have a high optical rotation: $[\alpha]_D^{21} + 500^\circ$; other properties were in agreement with those described by Curtin & Reilly (1940). The empirical formula considered to be in best agreement with all the analyses is $C_{21}H_{25}O_5Cl$, although $C_{21}H_{25}O_5Cl$ is not completely excluded. Curtin & Reilly (1940, correction) ascribed to sclerotiorin the formula $C_{20}H_{21}O_5Cl$ on the basis of one analysis.

With 1 molecule of 2:4-dinitrophenylhydrazine, sclerotiorin forms a compound $C_{27}H_{27(29)}O_8N_4Cl$ which is probably a hydrazone. With hydroxylamine in excess, 2 mol. combine with 1 of sclerotiorin to give $C_{21}H_{23(25)}O_4N_2Cl$ which contains 1 mol. of water less than is required for a dioxime. Sclerotiorin reacts immediately with 1 mol. of ammonia in the cold with loss of 1 mol. of water yielding $C_{21}H_{24(26)}O_4NCl$. This product, and the alkaline-hydrolysis acid, $C_{10}H_{16}O_2$, had previously been obtained by Dr R. F. Hunter but were not investigated in detail. It appears probable that sclerotiorin contains one reactive aldehyde or keto group capable of forming a hydrazone or oxime; in the reaction with the second molecule of hydroxylamine and with ammonia some type of heterocyclic ring formation may be involved.

When sclerotiorin is warmed with dilute alkali, hydrolysis occurs and a colourless optically active monobasic acid, $C_{10}H_{16}O_2$, m.p. 92–92.5°, can be isolated in almost quantitative yield assuming 1 mol. from 1 mol. of sclerotiorin. This acid is doubly unsaturated and, from the fact that it adds on maleic anhydride, the double bonds appear to be conjugated. The other fission products were amorphous and have not yet been further examined. Ozonolysis of the unsaturated C_{10} acid affords in

low yield an acid, $C_5H_6O_3$, which was identified as β -acetylacrylic acid, $CH_3CO.CH=CH.COOH$, by comparison with a synthetic specimen of the free acid and by comparing the respective 2:4-dinitrophenylhydrazones. An optically active aldehyde or ketone, $C_5H_{10}O$, also a product of ozonolysis, was isolated in good yield as the (+)-2:4-dinitrophenylhydrazone. Since this must have an asymmetric carbon it can have only one formulation namely $CH_3.CH_2.CH(CH_3).CHO$ which is 2-methyl-*n*-butanal, the aldehyde corresponding with the optically active (fermentation) amyl alcohol found in fusel oil. Badin & Pacsu (1945) have described the 2:4-dinitrophenylhydrazone of this compound; their values for melting point and optical rotation correspond closely with ours. On the basis of these facts the product of alkaline fission of sclerotiorin must have the constitution 4:6-dimethylocta-2:4-dienoic acid, $CH_3.CH_2.CH(CH_3).CH=C(CH_3).CH=CH.COOH$.

The reason for the low yield of β -acetylacrylic acid obtained on ozonolysis is no doubt due to the fact that it is susceptible to further oxidation at the double bond. In confirmation of this a small amount of a bisdinitrophenylhydrazone was isolated which, as a mixed melting point determination indicated, was in all probability derived from methylglyoxal. This would be one of the expected products of ozonolysis of β -acetylacrylic acid.

There is one discrepancy which remains to be explained. The C_{10} acid was found to yield 1.8 *C*-methyl groups per molecule whereas the postulated constitution has three such groups. The group $=CH.C(CH_3)=CH$ is stated by Kuhn & L'Orsa (1931) to give 85% of the theoretical value for 1 *C*- CH_3 group. No evidence was available for the effect of the other two methyl groups present in the radical $CH_3.CH_2.CH(CH_3)-$. 2-Methyl-*n*-butanol (racemic) was therefore examined and found to yield only 37% of the theoretical value for 2 *C*- CH_3 groups. The total acetic acid formed from the C_{10} acid should therefore be 0.74 + 0.85 mol. = 1.6 mol., which is in fairly good agreement with the 1.8 mol. actually found.

The constitution of one fission product of sclerotiorin has thus been determined; further work is in progress to establish the structure of the rest of the molecule.

EXPERIMENTAL

Organism. Five strains of *P. multicolor* Grigorieva-Manilova and Poradielova were examined. These were obtained from Dr K. B. Raper of the Northern Regional Research Laboratory, Bureau of Agriculture, Peoria, Illinois, U.S.A., under the catalogue numbers N.R.R.L. 764, N.R.R.L. 2058, N.R.R.L. 2059, N.R.R.L. 2060 and N.R.R.L. 2324. Strain N.R.R.L. 2058 was received by us in July 1948, the remainder in December 1949. All the strains except N.R.R.L. 2324 are mentioned in *A Manual of the Penicillia* (Raper & Thom, 1949).

Medium. The usual Czapek-Dox medium of the following composition was employed: NaNO_3 2.0 g., KH_2PO_4 1.0 g., KCl 0.5 g., $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g., $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g., glucose 50 g., water to 1 l. The medium was distributed in 1 l. conical flasks, 350 ml. per flask. The flasks were plugged with cotton wool and sterilized by steaming on three successive days. They were then inoculated from wort agar slope cultures and incubated at 24° for the prescribed period.

Characteristics of cultures. The five strains of *P. multicolor* (twenty flasks of each strain) were incubated for 17 days, when ten flasks of each strain were harvested. The remaining ten flasks of each strain were harvested after 24 days. The following appearances were noted.

N.R.R.L. 764. Considerable pigmentation and sporing (in some flasks only sparse). All heavily sporing flasks had a strongly curled mycelium forming an island in the centre of the culture fluid.

N.R.R.L. 2058. Pure white mycelium. Traces of colour round edges. Pale medium.

Isolation of sclerotiorin from mycelia. The mycelium from each group of flasks was ground in a mortar and extracted in a Soxhlet with light petroleum of b.p. 40–60°, the yield of solid crystalline material separating from the petrol being collected each day. The extraction was continued until the day's yield (6 hr.) was less than 1 g. The crude sclerotiorin extracted from the mycelium by petrol was orange-yellow in colour and had m.p. about 200°. The product from each strain was separately recrystallized once from methanol, and then consisted of bright yellow needles melting 3–4° higher than previously and in each case showing no depression when mixed with the sclerotiorin obtained by Hunter from *P. sclerotiorum*. The collected data are recorded in Table 1. The whole of the partially extracted mycelia was finally combined and re-extracted continuously with petrol to exhaustion. In this way a further 11.4 g. of sclerotiorin were obtained. The total yield of pure sclerotiorin obtained from the five strains was 30.0 g., although from one strain, N.R.R.L. 2058, the yield was nil. The best yield was ob-

Table 1. Data obtained on harvesting groups of ten flasks of *Penicillium multicolor* incubated at 24°

Strain N.R.R.L.	Time of growth (days)	pH of culture fluid	Glucose in culture fluid (%)	Wt. of dry mycelium (g.)	Soxhlet yield of sclerotiorine (g.)	M.p. of recrystallized sclerotiorin (°)	Further yield of sclerotiorin from combined mycelia (g.)
764	17	4.0	0.20	32.4	0.8	204–205	11.4
2058	17	3.7	0.81	42.2	Nil	—	
2059	17	4.0	0.60	37.7	3.6	202–203	
2060	17	4.4	0.71	41.6	6.3	204–205	
2324	17	4.9	0.63	32.8	2.7	205–206	
764	24	4.6	Nil	32.3	0.9		
2058	24	4.3	0.21	47.6	Nil		
2059	24	4.5	0.10	38.7	2.8		
2060	24	5.1	0.21	43.6	6.6		
2324	24	5.2	0.18	32.0	2.4		

N.R.R.L. 2059. Strong sporing and colour.

N.R.R.L. 2060. Colour strong, less sporing. Strong brown coloured reverse.

N.R.R.L. 2324. White, wrinkled mycelium, a few showing colour. Colour round edges, strong brown reverse.

At the termination of the incubation period the culture fluid was filtered from the mycelium, which was dried in a vacuum oven. Glucose was determined polarimetrically in the filtrate, pH by indicator papers.

Reactions of the culture solution with aqueous FeCl_3 at 17 days were as follows: N.R.R.L. 764, solution intense emerald-green colour, very heavy precipitate not highly coloured; N.R.R.L. 2058, nil; N.R.R.L. 2059, almost immediate very heavy precipitate, pale buff; N.R.R.L. 2060, fair reddish-orange colour and slight precipitate; N.R.R.L. 2324, deep reddish-purple colour, overnight buff flocculent precipitate. Similar, perhaps somewhat more intense, reactions were obtained at 24 days.

On acidification with HCl the culture fluid of strain N.R.R.L. 2324 alone gave an immediate turbidity followed by a crystalline deposit consisting of bunches of colourless needles apparently slightly less in amount after 24 than after 17 days.

No antibacterial activity was shown by the culture fluid of any strain against *Micrococcus pyogenes* var. *aureus* (Oxford H strain) or *Escherichia coli* (National Collection of Type Cultures R.6).

tained from strain N.R.R.L. 2060 where the crude yield was 15% of the weight of the dry mycelium, without taking into account the fact that the mycelium at this stage was not completely extracted. This is a great improvement on the yield of 2% obtained by Curtin & Reilly (1940) from *P. sclerotiorum*.

Isolation of metabolic products from culture fluid of strain N.R.R.L. 2324

The total filtrate from the ten flasks of strain N.R.R.L. 2324 incubated for 24 days gave a reddish-purple colour with aqueous FeCl_3 . It was acidified with 150 ml. of conc. HCl (diluted with 150 ml. of water) to render it about 0.5N. Almost colourless needles of *product I* were slowly deposited. After being chilled overnight the crystals were collected, washed with 0.5N-HCl and dried. The faintly yellow crystals, wt. 0.41 g. had m.p. 108°, raised on sublimation to 110.5°. They were somewhat soluble in cold, more readily in hot water. They were precipitated again from cold water on addition of HCl to 0.5N. The aqueous solution gave a cherry-red colour with FeCl_3 ; the compound did not contain halogen (Beilstein test). Found (Weiler): C, 58.5; H, 6.4%. No nitrogen present. $\text{C}_{11}\text{H}_{14}\text{O}_6$ requires C, 58.4; H, 6.2; $\text{C}_{13}\text{H}_{16}\text{O}_8$ requires C, 58.2; H, 6.0%. Equivalent by titration: 0.0275 g. required 1.78 ml. 0.1N-NaOH to neutralize to phenolphthalein; the end point was difficult to determine

owing to a yellow colour produced with alkali. Equivalent 146. $C_{11}H_{14}O_5$ (dibasic) requires 113. $C_{13}H_{16}O_6$ (dibasic) requires 134. When the titrated solution was acidified to 0.5N with HCl the original acid crystallized.

The filtrate from the acidified culture solution was extracted by shaking with ether (1.5 l. + 1.0 l. + 1.0 l.). On evaporation of the solvent 2.35 g. of a semi-solid residue remained. The extracted liquor after neutralization no longer gave a red-purple colour with $FeCl_3$. The ether extract was treated with successive portions of hot water. Crystals were obtained from the aqueous solution on cooling (0.31 g.) and a further amount (0.17 g.) on acidification of the mother liquor. These fractions had m.p. 123–124° with preliminary softening. A third fraction (0.35 g.) had m.p. 121°. Crystallization from ethyl acetate-benzene afforded pale yellow plates or leaflets consisting of *product II*, m.p. 130–131°. After sublimation in high vacuum the substance

also recorded in Table 2. The overall yields of reasonably pure sclerotiorin from the three batches (neglecting the cruder material obtained by concentration of the petrol used for extraction) amounts to 12.3% of the dry weight of the mycelium.

Sclerotiorin

A sample recrystallized from methanol was sublimed in high vacuum at 170–180°, bath temperature. The product had m.p. 205–206°, unchanged on a second sublimation.

Found (Weiler and Strauss): C, 64.6, 64.2, 64.2; H, 6.0, 6.1, 5.8; Cl, 10.2, 9.1, 9.2%; mol.wt. (Rast) 400, 393. (Found (Imperial Chemical Industries, Welwyn): C, 64.1, 64.6; H, 6.2, 6.2; Cl, 8.9, 9.0. Calc. for $C_{21}H_{25}O_5Cl$: C, 64.5; H, 5.9; Cl, 9.1%; mol.wt. 391. Calc. for $C_{21}H_{25}O_5Cl$: C, 64.2; H, 6.4; Cl, 9.0%; mol.wt. 393. Optical activity: $[\alpha]_{D}^{21} + 54.9^\circ$; $[\alpha]_D^{21} + 50.0^\circ$ (c, 1 in $CHCl_3$.)

Table 2. Data for two batches of strain N.R.R.L. 2060 and one of N.R.R.L. 2324 of *Penicillium multicolor*

Batch	Strain N.R.R.L.	No. of flasks	Period (days)	Final pH of culture medium	Dry wt. of mycelium (g.)	Yield of crude sclerotiorine		M.p. of crude product (°)	Recovered from evaporation of petrol (g.)	
						Final glucose concn. (%)	Wt. (g.)			% of dry mycelium
1	2060	101	18	4.6	411	0.64*	47.2	11.5	200–2	2.6
2	2060	100	20	4.5	379	0.40*	51.2	13.5	200–2	3.2
3	2324	100	18	5.0	345	0.52†	41.5	12.0	199–201	2.8

* By polarimeter.

† By Benedict method.

began to melt at 128°, clearing at 135°. Recrystallized from ethyl acetate, it shrank at 126°, softened at 128–132°, clearing at 133–134°. This behaviour appears to be characteristic and not due to impurity. The cherry-red $FeCl_3$ colour reaction in aqueous solution was almost identical with that obtained from product I. The substance contained no chlorine (Beilstein test) or methoxyl group. Found (Weiler and Strauss): C, 54.6; H, 6.3; $C_{13}H_{16}O_6$, requires C, 54.5; H, 6.3.

Unfortunately these two products, obtained from the culture filtrate, were only present in minute amount or absent from subsequent batches, so that they could not be further studied.

Accumulation of stock of sclerotiorin. Strain N.R.R.L. 2060, which had been selected from the preliminary experiment as affording the best yield of sclerotiorin was used for the preparation of a supply of the material. The details as to medium, method of culture and harvesting were the same as described for the preliminary experiment. The dry mycelium was ground in a mortar and extracted to exhaustion with light petroleum (b.p. 40–60°) in a large percolator with reflux condenser attached. The daily yield was filtered off and fresh petrol was used to continue the extraction. By evaporation of the petrol to small volume further recoveries of impure sclerotiorin from solution were made. The data for two batches of flasks worked up in this manner are recorded in Table 2.

In an attempt to obtain supplies of the products I and II from the culture fluid of strain N.R.R.L. 2324, one batch of 100 flasks was inoculated with this strain. The culture solution gave practically no $FeCl_3$ reaction and the desired products could not be isolated, but the yield of sclerotiorin from the mycelium was over 40 g. The data for this batch are

Derivatives

2:4-Dinitrophenylhydrazine. 2:4-Dinitrophenylhydrazine (0.20 g.) was dissolved in ethanol (25 ml.) and sclerotiorin (0.40 g., 1 mol. propn.) was added, followed by two drops of conc. HCl. The solution became dark red. It was refluxed for 20 min. and allowed to cool. Crystals (rods) had already begun to separate from the boiling solution. The crystals, intense dark red in colour, almost purple in bulk, were collected, washed with ethanol and dried *in vacuo*. The 2:4-dinitrophenylhydrazine, wt. 0.47 g., did not melt up to 360°. (Found (I.C.I. Welwyn): C, 57.0, 56.9, 56.8, 56.9; H, 5.3, 5.2, 4.9, 4.9; N, 9.5, 9.3; Cl, 5.8, 6.2, 6.2%. $C_{27}H_{27}O_8N_4Cl$ requires C, 56.8; H, 4.8; N, 9.8; Cl, 6.2. $C_{27}H_{29}O_8N_4Cl$ requires C, 56.6; H, 5.1; N, 9.8; Cl, 6.2%.)

Compound with hydroxylamine. Sclerotiorin (0.25 g.) was dissolved in ethanol (10 ml.) by warming, and hydroxylamine hydrochloride (0.25 g.) was added. The mixture rapidly darkened to an almost black liquid. It was refluxed for 0.5 hr. and allowed to cool. As there was no separation of crystals it was poured into water, and the bulky dark-red flocculent precipitate was collected, washed with water and dried. The product (0.26 g.) had m.p. 135–148°. After being twice recrystallized from methanol-water (4:1 v/v) the product (0.12 g.) was in the form of clusters of microplates, deep orange in colour, and appeared homogeneous. The product shrank and darkened from 164°, softened about 170° to a black tar, but there was no definite melting point. (Found (Weiler and Strauss): C, 62.9, 62.0; H, 6.4, 6.2; N, 7.0; Cl, 8.8. $C_{21}H_{25}O_5N_2Cl$ requires C, 62.6; H, 5.75; N, 7.0; Cl, 8.8; $C_{21}H_{25}O_5N_2Cl$ requires C, 62.3; H, 6.2; N, 6.9; Cl, 8.8%.)

Compound with ammonia. Sclerotiorine (1.0 g.) was dissolved by gentle agitation in conc. NH_3 (d, 0.880, 20 ml.). The deep red solution obtained (5 min.) was immediately diluted with water (100 ml.) and made faintly acid to Congo red with 2N- H_2SO_4 . The red precipitate was collected, washed with water and drained. Without drying it was crystallized from methanol (40 ml.)-water (20 ml.). The bright red needles obtained (0.85 g., m.p. 228°, to a black tar), were recrystallized from the same solvent. The product then had m.p. 228–229° (decomp.). (Found (I.C.I. Welwyn): C, 64.8, 64.9; H, 6.1, 6.2; N, 3.5, 3.6; Cl, 9.1, 9.2%. $\text{C}_{21}\text{H}_{24}\text{O}_4\text{NCl}$ requires C, 64.7; H, 6.2; N, 3.6; Cl, 9.1; $\text{C}_{21}\text{H}_{26}\text{O}_4\text{NCl}$ requires C, 64.3; H, 6.7; N, 3.6; Cl, 9.05%.)

Degradation

Alkaline hydrolysis. Isolation of C_{10} acid. Sclerotiorin (5 g., the crude petrol extract is quite satisfactory) was suspended in N-NaOH (500 ml.) and the mixture was warmed on the water bath to 50–55° with frequent shaking and maintained for about 0.5 hr. All the solid dissolved, forming a dark yellow-brown solution. The solution was cooled to 10° and 2N- H_2SO_4 (250 ml.) was added slowly with shaking and cooling so that the temperature did not rise above 15°. Then a slight excess of acid was added until the mixture was acid to Congo red. When the precipitate had settled the mixture was filtered. The solid was a light chocolate-brown powder when dry, wt. 2.63 g.

The solid was extracted with cold light petroleum (b.p. 40–60°, 4 × 50 ml.), filtering from the brown amorphous material. The crystalline orange-coloured residue (wt. 1.70 g.) left on removal of the petrol was recrystallized from methanol (20 ml.) + water (20 ml.) to give a slightly coloured product (1.55 g.) of m.p. 91–92°. This was dissolved in NaOH (0.4N, 25 ml.) and the solution was treated with decolorizing carbon (1 g.). On acidification with HCl the C_{10} acid formed a crystalline precipitate of colourless rods, m.p. 92–92.5°, from which chlorine was absent. (Found (Weiler and Strauss): C, 71.7; H, 9.1; C-CH_3 , 16.1%. $\text{C}_{10}\text{H}_{16}\text{O}_2$ requires C, 71.4; H, 9.6, mol.wt. 168, for 1 C-CH_3 , 8.9%, for 2, 17.9%, for 3, 26.8%.)

Equivalent. 0.5313 g. of the acid required 31.6 ml. of 0.1N-NaOH for neutralization to phenolphthalein, giving an equivalent of 168.

Optical rotation. $[\alpha]_{5461}^{20} + 82.7^\circ$, $[\alpha]_{5790}^{20} + 71.4^\circ$ (c, 4 in ethanol).

Derivatives of C_{10} acid

Hydrogenation. Adams's catalyst (PtO_2 , 0.1000 g.) was hydrogenated at room temperature in ethanol (50 ml.) and required 11.12 ml. of H_2 . The C_{10} acid (0.4965 g.) was then added and the hydrogenation continued. The uptake of H_2 was slow and practically ceased after 40 min. when 52.4 ml. (uncorr.) of H_2 had been absorbed. A further amount (0.1000 g.) of Adams's catalyst was added without prior reduction and the hydrogenation continued. The uptake was now rapid and reached constancy in 20 min. when a further 93.4 ml. (uncorr.) of H_2 had been absorbed (after allowing for catalyst uptake). The total uptake of H_2 at N.T.P. by the acid was 132 ml. For an uptake of four atoms of H 0.4965 g. of a compound $\text{C}_{10}\text{H}_{16}\text{O}_2$ requires 132 ml.

The solution was filtered from the catalyst and made up to 100 ml. with ethanol for determination of optical rotation, using a 4 dm. tube. $[\alpha]_{5461}^{20} + 22.0^\circ$ in ethanol (c, 0.5).

On evaporation of the ethanol *in vacuo* a pale amber-coloured oil, consisting of the C_{10} tetrahydro acid, remained (0.494 g.). It was dried to constant weight over P_2O_5 for analysis. (Found (Weiler and Strauss): C, 69.9; H, 11.6; $\text{C}_{10}\text{H}_{20}\text{O}_2$ requires C, 69.7; H, 11.7% mol.wt. 172.)

Equivalent. The hydrogenated acid (0.4137 g.) in 5 ml. of ethanol was titrated with 0.1N-NaOH. It required 22.70 ml. (corr. for ethanol blank). Equivalent, 182, indicating a monobasic acid if $\text{C}_{10}\text{H}_{20}\text{O}_2$.

p-Bromophenacyl ester. The above neutralized solution was evaporated *in vacuo* to about 5 ml. and refluxed with p-bromophenacylbromide (0.634 g.) in ethanol (30 ml.) for 1 hr. A trace of sediment was filtered off. On chilling the solution, crystals (shining leaflets) appeared which redissolved at room temperature. The chilled solution was filtered quickly giving 0.20 g. of plates, m.p. 40–55°. The product was dissolved in light petroleum, filtered from a little insoluble matter and recrystallized from 90% aqueous methanol, giving colourless plates, m.p. 41°, of the p-bromophenacyl ester. (Found (Weiler and Strauss): C, 58.8; H, 6.8; Br, 21.3; $\text{C}_{18}\text{H}_{25}\text{O}_3$ Br requires C, 58.55; H, 6.8; Br, 21.6%.)

Diels-Alder product from C_{10} acid. The C_{10} acid (0.3 g.) and maleic anhydride (0.2 g.) were fused together (without solvent) and maintained at 100–110° for 2 hr. The viscous oily product was taken up in a little dry ether. On evaporation of the ether a crystalline mass remained. This was redissolved in dry ether, filtered, and an equal volume of light petroleum (b.p. 40–60°) was added. Crystals of the addition product slowly deposited, of m.p. 144–152°, after three recrystallizations 147–148°, and on final sublimation 149–150°. (Found (Weiler and Strauss): C, 62.4; H, 6.5; $\text{C}_{14}\text{H}_{18}\text{O}_5$ requires C, 63.1; H, 6.8.) This indicates that the acid contains a conjugated double-bond system.

Degradation of C_{10} acid by ozonolysis

The C_{10} acid (0.50 g.) was dissolved by shaking in water (50 ml.) containing NaHCO_3 (0.50 g.). Ozonized oxygen was passed through the solution at room temperature, the outflow being bubbled through Brady's reagent (2.4-dinitrophenylhydrazine (0.3%) in 2N-HCl). A yellow precipitate began to form in the bubbler and the reaction mixture became slightly turbid. The ozone was stopped after 2.5 hr. but the O_3 stream was continued for a further 1 hr. After standing overnight, the pH was 8.0–8.4. The solution was extracted with ether (2 × 25 ml.). The ether extract gave only a small amount of material precipitable with Brady's reagent; this was not further examined. Air was bubbled through the aqueous layer to remove traces of ether and then N-HCl (5.95 ml.) was added to take up all the alkali. The white crystalline precipitate produced (0.07 g.) had m.p. 92–93° and was unchanged C_{10} acid.

The filtrate from the acid was evaporated *in vacuo* and the distillate was titrated. The operation was repeated after addition of water (50 ml.) up to four times in all. The titrated volatile acids amounted to 24.83 + 5.20 + 0.98 + 0.32 = 31.3 ml. of 0.1N. The non-volatile residue in the flask was titrated and found to require 5.31 ml. of 0.1N-NaOH. The equivalent amount of HCl was added and the solution was evaporated to dryness *in vacuo*. The residue was refluxed for a few minutes with 100 ml. then 50 ml. of ether. On evaporation of the ether a crystalline solid mixed with oil was obtained, of wt. 0.08 g. The mixture was fractionated from ether-light petroleum. Some of the fractions were oily, but from the best fraction about 20 mg. were picked out and

sublimed at water-pump vacuum. This product shrank slightly at 110°, softened at 120–122° and melted at 123–124°, showing a crystalline sublimate in the melting point tube. (Found (Weiler and Strauss): C, 52.2; H, 5.1; calc. for $C_5H_6O_3$: C, 52.6; H, 5.3 %.) A sample of β -acetylacrylic acid was prepared synthetically from laevulic acid via β -bromo-laevulic acid according to the method of Wolff (1891). After crystallization from benzene and sublimation at water pump vacuum the synthetic product showed the following behaviour in the melting point tube: shrinks 115–120°, softens 122°, melts 124–125°. A mixture of the ozonolysis and synthetic acids behaved as follows: shrinks 110°, softens 122°, melts 124–125°. From the fact that there was no depression the conclusion was drawn that the acid produced by ozonolysis was acetylacrylic acid. This conclusion was confirmed by preparation of the dinitrophenylhydrazone.

2:4-Dinitrophenylhydrazone of ozonolysis acid. The residues from the crystallization of the above acid were dissolved in water and treated with Brady's solution (50 ml.). After 2 days, the bright yellow precipitate was collected and dried. Wt. 0.06 g., m.p. 263–264° (decomp.). On recrystallization from dioxan crystalline aggregates consisting of microrods of the 2:4-dinitrophenylhydrazone separated (0.03 g.), m.p. 263–264° (decomp.), darkening and shrinking from 260°. (Found (Weiler and Strauss): C, 45.0; H, 3.5; N, 19.2; calc. for $C_{11}H_{10}O_8N_4$: C, 44.9; H, 3.4; N, 19.1 %.) A sample of the 2:4-dinitrophenylhydrazone was prepared in like manner to the above from synthetic β -acetylacrylic acid. After recrystallization from dioxan it had m.p. 263–264° (decomp.) with darkening and shrinking from 260°. A mixture of this substance with the dinitrophenylhydrazone derived from the ozonolysis product showed no depression in melting point.

2:4-Dinitrophenylhydrazone in bubbler. The yellow solid which had been precipitated in the bubbler containing Brady's reagent was collected (wt. 0.44 g.) and recrystallized first from ethanol and then twice from light petroleum (b.p. 80–100°), filtering from a little insoluble matter. The melting point of the main product was then constant at 133.5°. (Found (Weiler and Strauss): C, 49.8; H, 5.3; N, 21.0; calc. for $C_{11}H_{14}O_4N_4$ (from $C_5H_{10}O$): C, 49.6; H, 5.3; N, 21.0 %.)

Optical activity: $[\alpha]_{5461}^{19.0} + 44.2^\circ$, $[\alpha]_{5790}^{19.0} + 38.0^\circ$ (c, 1 in acetone). Badin & Pacsu (1945) give for the 2:4-dinitrophenylhydrazone of (+)-2-methylbutanal-1 the values: m.p. 132.5–133°, $[\alpha]_D + 32.1^\circ$ in acetone (c, 4.99). The petrol-insoluble material from the bubbler was an orange powder.

The amount was too small for recrystallization. It gave an intense blue-violet colour when dissolved in ethanol and treated with a drop of dilute NaOH indicating that it was a bisdinitrophenylhydrazone. It had m.p. 300° (decomp.) and showed no depression when mixed with authentic methylglyoxal bis 2:4-dinitrophenylhydrazone.

Carbon-methyl determination on 2-methylbutanol. The determination was made on a sample of racemic 'active amyl alcohol' (β -methylbutanol) obtained from British Drug Houses. (Found (Weiler and Strauss): C-CH₃ 12.5 %. Theor. for $C_5H_{12}O$ (with 2 C-CH₃): C-CH₃ 34.0 %). The value found is thus 37 % of theoretical.

All melting points are uncorrected.

SUMMARY

1. Five strains of *Penicillium multicolor* Grigorieva-Manilova and Poradielova were investigated. From the mycelium of four of these strains sclerotiorin was isolated in considerably higher yield than had been obtained by Curtin & Reilly (1940) from the mycelium of *P. sclerotiorum* van Beyma.

2. From the analysis of sclerotiorin itself and its derivatives the most probable empirical formula is $C_{21}H_{23}O_5Cl$. It has $[\alpha]_D^{21} + 500^\circ$ in $CHCl_3$ (c, 1).

3. When sclerotiorin is warmed with dilute aqueous sodium hydroxide it is hydrolysed giving a crystalline doubly unsaturated monobasic acid, $C_{10}H_{16}O_2$. The acid gives rise to (+)-2-methyl-*n*-butanal and β -acetylacrylic acid on ozonolysis, indicating that the acid has the constitution (+)-4:6-dimethylocta-2:6-dienoic acid.

4. In addition, two new products were isolated from the culture fluid of one strain of the fungus. These two substances, product I, m.p. 110.5°, and product II, m.p. 133–134°, after softening at 128–132°, both gave in aqueous solution a cherry-red colour with ferric chloride. From the analyses probable empirical formulae were deduced as follows: product I, $C_{13}H_{16}O_6$; product II, $C_{13}H_{18}O_7$.

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