Chemical Composition of a Sample of Mycelium of *Penicillium griseofulvum* Dierckx

By W. R. SMITHIES

Rothamsted Experimental Station, Harpenden, Herts

(Received 18 October 1951)

Both fungal and bacterial processes occur in the formation of soil organic matter (Waksman, 1938), which is itself composed, at least in part, of living micro-organisms and those parts of their remains which are resistant to chemical and enzymic decomposition. A knowledge of the composition of micro-organisms should, therefore, help to shed some light on the constitution of soil organic matter. Whilst much work has been done on the isolation and characterization of low molecular weight constituents of fungi, there are no analyses of mycelium in which the various macromolecular compounds are well defined.

A variety of carbohydrate preparations have been isolated from fungal tissue (Norman, Peterson & Houtz, 1932), and it has long been recognized that the cell wall of many fungal species is chitinous (Norman & Peterson, 1932; Brian, 1949). Protein concentrates have been extracted by Gorgica, Peterson & Steenback (1934) and Bohonos, Woolley & Peterson (1942). Takata (1929), and Akasi (1939) isolated nucleic acid preparations. Claims have been made for the isolation of 'crude lignin' or 'lignin-like complexes' by Thom & Phillips (1932), Phillips (1938) and Pinck & Allison (1944). Porges (1932) and Schulz (1937) studied the effect of cultural conditions on mycelial composition but, for the most part, their fractions are quite arbitrary and sometimes misleading. The literature is summarized and discussed by Foster (1949).

In this paper various analytical methods have been studied from the point of view of their usefulness in the determination of the chemical components, particularly the macromolecular ones, of a sample of mycelium of *Penicillium griseofulvum* Dierckx, grown as a surface pad. This is not a normal or important components of the soil flora, but was chosen for this work, which will be used as a model for future investigations on the predominant component of the soil, because it was readily available.

No precautions were taken to separate spores, or to avoid autolysis, and values given are by no means claimed to be typical of mould mycelium.

MATERIALS AND GENERAL METHODS

Culture of mycelium. I am indebted to Mr P. J. Curtis of the Butterwick Research Laboratories of Imperial Chemical

Industries for the following information. Culture: *P. griseofulvum* Dierckx (BRL 374) (ex Prof. H. Raistrick no. P 38). Medium: Czapek-Dox (Raistrick), containing 7.5% crude glucose and 0.1% peptone. The mould was grown in earthenware culture vessels of the type described by Abraham, Chain, Fletcher, Gardner, Heatley, Jennings & Florey (1941) using 1 l. medium in each and an incubation period of 14 days.

Preparation of mycelium. The mycelium, received a few hours after harvesting, was spread thinly on sheets of paper, and by the following day most of the moisture had evaporated, but although allowed to dry at room temperature for over a month, it never became sufficiently crisp to powder satisfactorily in a small C. and N. (Christy & Norris, Chelmsford) laboratory mill (0.5 mm. sieve). However, there was no difficulty with mycelium that was dried thus for only 3 or 4 days, if placed in the hot room at 40° for several hours before milling.

A portion of the powdered mycelium (*M*) was exhaustively extracted in a Soxhlet apparatus with light petroleum (b.p. 40–60°), ether, ethanol, acetone and chloroform, in that order, and again dried in air (*Ms*). Subsequently it was found that acetone, ethanol and chloroform alone were as effective and light petroleum or ether extracted no more material.

A portion of *Ms* was extracted twice with cold water (10 vol. for 30 min. with stirring) and then three times with the beaker standing in a boiling-water bath. The residual mycelium was exhaustively extracted with ethanol and air-dried (*Msw*).

Dry matter. Weighed samples were dried at 100–105° overnight and reweighed.

Total nitrogen. This was determined by a micro-Kjeldahl method using $\text{SeO}_3 \cdot \text{CuSO}_4 \cdot 5\text{H}_2\text{O} : \text{K}_2\text{SO}_4 : 1 : 1.8$ as catalyst.

NH₃ nitrogen. This was estimated by microdiffusion in a Conway dish followed by nesslerization (Conway, 1947).

α -Amino-acid N was determined by the ninhydrin-titration method of Van Slyke, Dillon, MacFadyen & Hamilton (1941) at pH 2.5 and a reaction time of 8 min.

Total P. This was estimated by a modification of the method of Kuttner & Lichtenstein (1932).

Carbohydrate. This was determined by the colour given on heating with orcinol in H_2SO_4 using a glucose standard (Pirie, 1936). The intensity of colour was measured with a photoelectric colorimeter (the Evans Electroselenium Portable model, using Ilford filter 622) and the sugar concn. was 15–100 μg . in a final volume of 10 ml. Equal weights of hexoses give values of 100%, of polyhexoses, 110% (cf. Holden, Pirie & Tracey, 1950). No interference by protein (gelatin), glucosamine hydrochloride, or mannitol was detectable when 50 μg . of these substances were added to 50 μg . of glucose.

Reducing substances. These were determined by Nelson's

Table 1. *Composition of mycelium preparations (moisture free)*

Mycelium	Yield (mg./g. <i>M</i>)	Composition (mg./g. mycelium)			
		Ash	N	P	Carbohydrate (as polyhexose)
<i>M</i> —whole mycelium	—	38	31.5	7.0	510
<i>Ms</i> —extracted by organic solvents	700	34	35	7.6	640
<i>Msw</i> —extracted by organic solvents and by water	630	14	34.5	3.3	700

(1944) adaption of the Somogyi method, without the preliminary precipitation of protein.

Amino sugar estimations. These were made on hydrolysates freed from the HCl used by repeated distillation *in vacuo*. The method of Elson & Morgan (1933) was modified by Mr M. V. Tracey of this department according to Blix (1948) and Immers & Vasseur (1950). Amino sugar was also determined by distillation with saturated Na_3PO_4 solution plus sodium borate, and estimation of the NH_3 produced by nesslerization (Tracey, 1951).

Paper chromatography. One-dimensional, descending paper chromatography was used for qualitative and quantitative examination of the carbohydrate components (Flood, Hirst & Jones, 1948). The sugar spots were developed with either ammoniacal AgNO_3 (Partridge, 1948) or aniline phthalate in glacial acetic acid (Hough, Jones & Wadman, 1950).

Colloidal material. This was isolated by dialysis in a cellophan membrane into frequently changed distilled water.

EXPERIMENTS AND RESULTS

In general, estimations were carried out, not only on whole mycelium (*M*), but also on mycelium that had been exhaustively extracted with organic solvents (*Ms*) and then with cold and hot water followed by a further extraction with ethanol (*Msw*) (cf. Table 1). Whether or no extracted material interfered with the determinations could be recognized.

Organic solvent extraction. This removed 31% of the dry weight of *M*. In the preparation of *Msw*, further extraction with ethanol removed another 1% of the dry weight of *M*. A little more fat-soluble material was liberated by hydrolysis of *Msw* with *N*-HCl in a boiling-water bath for 2 hr. Amounting to 2.5% of the dry weight of *M*, it was extracted from the solid residue by boiling ether-ethanol mixture (1:1) and from the aqueous layer by ether. More severe conditions of hydrolysis or refluxing over a flame produce traces of black 'humin' material which is itself soluble in ethanol. A total of 34.5% of the dry weight of *M* is, therefore, actually or potentially extractable from the mycelium with organic solvents.

No attempt was made to prepare pure compounds from these extracts, with the exception that mannitol was readily isolated by extraction with water, and after recrystallization had m.p. 165°, unchanged when mixed with an authentic specimen. The hexaacetate had m.p. 120°, also unchanged on admixture with an authentic specimen.

The combined material extracted with organic solvents contained 22% of the total N of mycelium *M*, and 24% of the total P.

Water extraction (cf. Tables 2a and b). Mycelium *M* (1 g.) extracted four times with water (25 ml.) in a steam bath, lost 29% of its dry weight. On dialysis 15% of the dry matter of the extract was retained as colloidal material. The extraction of mycelium *Ms* yielded dry matter equivalent to

Table 2a. *Composition of water extract of mycelium (M)*

	Content		
	mg./g. dry matter	Percentage of total constituent in <i>M</i>	Percentage of each constituent in colloidal fraction
Nitrogen	35	32	19
Carbohydrate (as glucose)	220	10	36
Reducing substance (as glucose)	20	—	—

Table 2b. *Composition of water extract of solvent-extracted mycelium (Ms)*

	Content		
	mg./g. dry matter	Percentage of total constituent in <i>M</i>	Percentage of each constituent in colloidal fraction
Nitrogen	40	7	75
Phosphorus	55	45	20
Carbohydrate (as glucose)	330	3	100

Table 3. *α-Amino-acid N content of HCl hydrolysates of mycelium*

(Mycelium taken into 12*N*-HCl solution, was diluted with an equal volume of water, and heated in a sealed tube immersed in boiling water, for 20 hr. The HCl was removed by vacuum distillation.)

Mycelium	α-Amino-acid N (mg./g. N of mycelium)	α-Amino-acid N (mg./g. N of mycelium <i>M</i>)
<i>M</i> —whole mycelium	46	46
<i>Ms</i> —extracted by organic solvents	56	44
<i>Msw</i> —extracted by organic solvents and by water	58	40

only 5.5% of the dry weight of mycelium *M*. On dialysis, 50% of the dry matter of the extract was retained.

Protein

Results are summarized in Table 3. A protein concentrate which was free, as far as could be ascertained, from other nitrogenous material was prepared by extraction of *Ms* with cold 2*N*-NaOH, precipitation of the carbohydrate with an equal volume of ethanol and, after bringing to pH 7 with glacial acetic acid, removal of the bulk of the ethanol by

distillation *in vacuo*. The small amount of insoluble material was spun off and contained less than 2% N. Trichloroacetic acid solution (20%) was added to the filtrate to a final concentration of 5% and the precipitate centrifuged off and washed with ethanol. About 0.04 g. of a pale-brown powder containing 9% N was thus isolated from 1 g. of *Ms*. After acid hydrolysis 720 mg. α -amino-acid N/g. total N was found. The apparent amino-sugar content, determined by the Elson & Morgan method was less than 1%, while in the case of Tracey's (1951) method, the $\text{NH}_3\text{-N}$ (50 mg./g. total N) was identical with the N given off as ammonia on distillation with saturated sodium phosphate solution. How far this figure of 720 mg. α -amino-acid N/g. total protein N is representative of the mycelial protein as a whole is not known, but under the conditions of hydrolysis used it is fairly typical of other protein preparations examined.

Assuming, therefore, that the mycelial protein of *M* yields 440 mg. α -amino-acid N/g. total N, and this value represents 72% only of the total protein N, then about 60% of the total N of mycelium *M* must be present in protein, which, using the conventional conversion factor 6.25, corresponds to 12% of protein in mycelium *M*.

Nucleic acid

An estimation of nucleic acid N and P was made following the method of Schneider (1945). Trichloroacetic acid solution (12.5 ml. of 10%) was added to mycelium *Ms* or *Msw* (1 g.) which had been allowed to soak in water (5 ml.) at room temperature for 30 min., and the mixture stirred for 20 min. After filtering with suction through madapolam the residue was refluxed, first with ethanol, then with chloroform. The air-dried product was then extracted with 5% trichloroacetic acid (25 ml.), with the extraction tube standing in boiling water, for 15 min. After filtering and washing, first with 5% trichloroacetic acid and then with water, extract and washings were combined. The cold trichloroacetic acid removed little more N and P from *Ms* than did water, and the extract from *Msw* was very slight. With hot trichloroacetic acid the amount of P extracted amounted to 110 mg./g. of total P in *M*, and of N to 70–100 mg./g. of the total N in *M*. Accepting the values found for phosphorus extracted, and assuming that nucleic acid contains about 10% P, this leads to a value of 8.0 mg. of nucleic acid/g. mycelium *M*.

After hydrolysis of *Ms* with $\text{n-H}_2\text{SO}_4$ for 7 hr. in a sealed tube immersed in boiling water, ribose was detected in the hydrolysate by paper chromatography. Quantitative measurements using a known amount of xylose as standard showed the presence of about 1.0 mg. ribose/g. *M*.

Chitin

The presence of polyacetylglucosamine in the mycelium (10 g.) was shown by hydrolysis with 6N-HCl at 100° for 6 hr. of the fraction of the mycelium remaining after ex-

traction with 2N-NaOH. Following distillation of the acid *in vacuo*, glucosamine hydrochloride was isolated from the solid residue by rubbing with acetone, followed by two crystallizations from aqueous ethanol. The colourless crystals (0.3 g.) had $[\alpha]_D^{20} + 71^\circ$ (c, 1.5 in water), after standing. An authentic sample of glucosamine hydrochloride gave $[\alpha]_D^{20} + 72^\circ$ (c, 1.5 in water), after standing. (Found N, 6.6. $\text{C}_6\text{H}_{14}\text{O}_6\text{NCl}$ requires N, 6.5%.) The osazone was identical in crystalline form with glucosazone prepared from an authentic specimen of glucosamine hydrochloride, and had m.p. and mixed m.p. 204–205°.

A mixture of the mycelium (10 g.) with 72% (w/w) H_2SO_4 (20 ml.) after standing for 2 days was diluted to 700 ml. and refluxed for 10 hr. The acid was partially neutralized with $\text{Mg}(\text{OH})_2$ and the product steam distilled. A definite positive test for acetic acid with lanthanum chloride and iodine (Krüger & Tschirch, 1930) was not given by this distillate, but when volatile reducing substances were fixed by oxidation with KMnO_4 beforehand, the steam distillate then gave a positive test. This effect is presumed to be due to the sulphur dioxide present in the hydrolysate, since sulphites were shown to reduce the sensitivity of the colour reaction. A small amount of a colourless, crystalline, water-insoluble material, which has not been identified, was filtered from the main bulk of the distillate which, after neutralization with NaOH was evaporated to small volume, acidified with HCl and extracted with ether. After evaporation of the ether, the extract smelt strongly of acetic acid and gave a *p*-phenylphenacyl ester, which after recrystallization had m.p. 109°. An authentic sample prepared from acetic acid had m.p. 110° and the mixed m.p. was 109°.

Adapting the method used by Black & Schwartz (1950) for the estimation of chitin in crawfish waste, mycelium (1 g.) was extracted once with cold 5% (w/v) NaOH (25 ml.) by standing, with frequent stirring, for 30 min. The insoluble material, spun down or filtered through madapolam under suction was extracted three times with the beaker standing in boiling water. Further extraction removed only traces of N. The residue was washed with water, *n*-acetic acid, again with water, refluxed with ethanol, followed by acetone, then chloroform, and air dried. Table 4 gives yields and analyses of the product, *M.alk.* The cold alkaline extract was used as a source of alkali-soluble carbohydrate.

After hydrolysis of *M.alk.* or chitin itself with 6N-HCl for 16 hr., no α -amino-acid N was found. Assuming, therefore, that the N content of *M.alk.* is entirely derived from amino-sugar residues, a minimum of about 9% of the total N of mycelium *M* is present in this form. This corresponds to about 19% of chitin in *M.alk.* and to about 4% in mycelium *M*.

Hydrolysis of mycelium with HCl, removal of the acid by distillation *in vacuo*, and estimation of amino sugar, gave further information. Initial experiments were carried out on an authentic sample of chitin (100 mg.) brought into 12N-HCl solution (1 ml.) by standing at room temperature, then

Table 4. Alkali-resistant residue *M.alk.*

Mycelium source	Yield (mg./g. of mycelium source)	Yield (mg./g. of mycelium <i>M</i>)	Nitrogen content	
			(mg. N/g. <i>M.alk.</i>)	mg. N in <i>M.alk.</i> /g. N in mycelium <i>M</i>
<i>M</i> —whole mycelium	230	230	13	84
<i>Ms</i> —extracted by organic solvents	320	220	13	90

The above figures refer to moisture-free material

diluting and heating in a sealed tube in boiling water. Amino-sugar content was estimated by Tracey's (1951) method. Heating with 6*N*-HCl for 3–15 hr. gave values between 86 and 92% of the total N given off as NH₃, without a distinct maximum; 4*N*-HCl for 10 hr. gave about 75% of the total N as NH₃, and 3*N*-HCl for 10 hr. gave about 70%. Irving (1909), on polarimetric evidence, claimed that chitin was completely hydrolysed by conc. HCl in 8–10 hr. at 40–45°. However, chitin hydrolysed with 10*N*- or 12*N*-HCl for 10 hr. at 37° gave only 40% of its N as NH₃ when distilled with sodium phosphate. Even after 48 hr. hydrolysis, no more than 50% was obtained.

On the basis of these results, hydrolysis of mycelium was carried out by taking up the sample (100 mg.) in 12*N*-HCl (1 ml.), diluting after 2–3 days (when the mycelium was almost completely dispersed) with an equal volume of water and heating for 6 hr. in a sealed tube in boiling water. A small amount of black, insoluble material was formed, and care was taken to carry out estimations by the Elson & Morgan method on the clear, pale yellow or brown supernatant liquid.

In the case of mycelium hydrolysates the N volatile on distillation with sodium phosphate solution includes a proportion of NH₃-N derived from the degradation of amino-acids and this must be allowed for. However (Table 5) about 20% of chitin N is also degraded to NH₃ and hence the net amounts of N in column 3 are taken to represent only 80% of the total chitin N. A correction is made for this in column 4. About 12% of the total N of mycelium *M* occurs in amino-sugar residues, corresponding to 5.5% chitin in the mycelium.

Discordant results are obtained unless the laboratory atmosphere is clear. The size of the blanks are reduced if the steam source is made alkaline. Under these conditions the following results were obtained:

N-Acetylglucosamine. (Found: CH₃CO, 19.3. Calc. for C₈H₁₃O₆N.COCH₃: CH₃CO, 19.4%.)

A chitin sample. (Found: N, 6.0; CH₃CO, 19.2. Calc. for anhydroacetylglucosamine, C₈H₁₀O₄N.COCH₃: N, 6.9; CH₃CO, 21.2%.)

M.alk. (Found: CH₃CO, 4.1. Calc. for a chitin content of 19%: CH₃CO, 4.0%.)

*M*s. (Found: CH₃CO, 1.7, corresponding to 1.2% CH₃CO in mycelium *M*.)

*M*sw. (Found: CH₃CO, 1.75, corresponding to 1.1% CH₃CO in mycelium *M*.)

M. (Found: CH₃CO, 1.5%.)

If mycelium *M* contains 5.5% of chitin, it would have 1.2% CH₃CO. The higher percentage found in *M* itself must be due to the presence of acetyl compounds or other materials giving rise to volatile acid on hydrolysis, among the fat-soluble components. No higher fatty acids than acetic were detected in the distillate by paper chromatography using the method of Brown (1950). The titration figure was not reduced by the addition of KMnO₄ before distillation, confirming the absence of formic acid.

Carbohydrate

To estimate the carbohydrate content of mycelium by the orcinol method (Pirie, 1936), which obviates interference from protein or amino-sugar, the sample is taken up in 72% (w/w) H₂SO₄ (4–6 mg./ml.) by allowing to stand at room

Table 5. *Glucosamine N in hydrolysates of mycelium*

(Hydrolysis by 6*N*-HCl for 6 hr. Results as mg./g. total N in material except in last two columns.)

Material	Ammonia N (AN)	Distilled N (DN)	DN - AN	(DN - AN) × 1.25	N (E & M)	Total N in mycelium <i>M</i> (mg./g.)	
						(DN - AN) × 1.25	N (E & M)
Glucosamine HCl	50–60	960–1050	900–1000	—	96–106	—	—
Chitin	70–80	870–920	780–850	—	93–110	—	—
Mycelium <i>M</i>	80–90	180–190	90–110	120	90–110	120	100
Mycelium <i>M</i> s	60–70	190	120–130	150	130–140	117	100
Mycelium <i>M</i> sw	50–70	190–220	140	175	180	122	125
<i>M</i> .alk.	30–60	800–850	790–820	1000	720–870	—	—

N (E & M) refers to glucosamine N estimated by Elson & Morgan's (1933) method.

Distilled N refers to glucosamine N estimated by distillation with saturated sodium phosphate (Tracey, 1951).

Estimations of acetyl content were made by allowing the mycelium (50 mg.) to stand for several days in 72% (w/w) H₂SO₄ (0.5 ml.), diluting to 12.5 ml. (to give an approximately *N*-acid solution) and heating in a boiling-water bath for 3 hr. After unsealing, the tubes were allowed to stand uncorked for about an hour to free the solution from volatile inorganic acid. No acetic acid is lost. 1 ml. of the solution, mixed with about 4 g. MgSO₄·7H₂O (to accelerate the distillation of the acid; Olmsted, Whitaker & Duden, 1929), was distilled in a Markham (1942) still at the rate of about 1 drop/sec., until exactly 10 ml. of distillate had been collected. The distillates were titrated with 0.2*N*-Ba(OH)₂ solution from a Conway microburette. Blank distillations were made from 1 ml. *N*-H₂SO₄ and 4 g. MgSO₄·7H₂O.

temperature for 1–3 days, when the solution from fat-free samples is completely clear and yellow to brown in colour. Mycelium *M* gives an opalescent solution due to acid-insoluble fatty components. The estimation is carried out at a suitable dilution. The values obtained were unaffected by the time the mycelium was allowed to stand in acid, up to 7 days.

The polyhexose content of *M* is taken as 45%, calculated from the value obtained for *M*s (Table 1). A certain amount of orcinol-positive material is therefore extracted by organic solvents, and this is presumed to be identical with the dialysable carbohydrate extracted by water from *M* (Table 2*a*). Although this water extract contains a small amount of reducing substance, no reducing sugar was

detected in it or the ethanolic extract by paper chromatography. After hydrolysis of a sample of ethanolic extract with $N-H_2SO_4$ glucose was detected by paper chromatography.

Only a small amount of colloidal carbohydrate is extracted by water from *M* or *Ms* (Tables 2*a* and *b*), but larger amounts are dispersed by cold 2*N*-alkali. By precipitation of the alkaline solution with an equal volume of ethanol (Norman & Peterson, 1932) a predominantly carbohydrate material is obtained representing 10–15% by weight of *M* and containing only 0.6–1.0% N. On hydrolysis with $N-H_2SO_4$ for 10 hr. in a sealed tube in boiling water, it was not taken entirely into solution, which contained only 600 mg. of reducing sugar (as glucose)/g. polysaccharide. On hydrolysis for 20 hr., after preliminary solution in 72% (w/w) H_2SO_4 , 850 mg. reducing sugar/g. of polysaccharide were obtained.

The alkali-extracted mycelium, *M.alk.*, contains about 74% of carbohydrate (as polyhexose), associated with chitin.

Ms, taken up in 72% (w/w) H_2SO_4 by allowing to stand for 2 days, diluted to give an *N*-acid solution, and hydrolysed for 20 hr. gave 73% of its dry weight as reducing substance, calculated as glucose. Paper chromatography indicated the presence of glucose, galactose, and mannose, approximately in the ratio 10:1.7:1, and these three sugars were also present in *M.alk.*, whereas the alkali-soluble polysaccharide contained only glucose. An osazone was prepared from a hydrochloric acid hydrolysate of this material (after removal of acidic and basic compounds with resin columns), identical in crystalline form, melting point and mixed melting point with an authentic specimen of glucosazone. Under conditions which readily yielded cellobiose octaacetate from cotton-wool cellulose, no such compound could be isolated from this alkali-soluble glucose polymer.

The presence of galactose in the mycelium was confirmed by oxidation with HNO_3 of an *HCl* hydrolysate of *Ms* (3 g.) freed from acidic and basic material, and isolation of mucic acid, m.p. 211–212° (decomp.); mixed melting point with an authentic specimen 212–213° (decomp.).

DISCUSSION

The preceding results lead to the figures for the composition of the mycelium given in Table 6. There is no indication that the mycelium contains 'lignin-like substances', and it is quite erroneous to suppose (as did Pinck & Allison, 1944) that the mycelium fraction insoluble after standing for 2 hr. in 72% (w/w) H_2SO_4 followed by refluxing after dilution to 3%, can be characterized as crude lignin. Even *M.alk.*, which certainly after alkali extraction contains no lignin, requires at least 24 hr. to disperse completely. On dilution of the clear solution and refluxing, a small amount of black material is formed but this is an invariable artifact

in the hydrolysis of material containing protein or amino sugar and carbohydrate.

Table 6. *Composition of mycelium M (moisture-free)*

(The air-dried mycelium contains in addition, about 10% moisture.)

	mg./g. <i>M</i>
Fat and organic solvent-soluble material	345
Residual, dialysable, water-soluble material	30
Polyhexose carbohydrate	450
Protein	120
Chitin	55
Nucleic acid	10
Residual, unextracted ash	10
Total	1020 mg.

Nitrogen is distributed among the organic solvent extract (22%), residual water-extractable and dialysable material (5%), protein (60%), chitin (12%), and nucleic acid (4%).

About 70% of the total phosphorus is extracted by organic solvents and water and 10% remains in *M.alk.* (which surprisingly contains 0.3% P). Nearly 10% is present in nucleic acid.

The composition of *M.alk.* approximates to: polyhexose 74%; chitin 19%; ash, 1%. The remainder has not been accounted for.

SUMMARY

1. The composition of a sample of mycelium of *Penicillium griseofulvum* has been studied with particular reference to the high molecular weight components.

2. Fats and compounds soluble in organic solvents amount to about 35%.

3. Protein (about 12%) was estimated by a determination of α -amino-acid nitrogen on various fractions hydrolysed with hydrochloric acid.

4. Carbohydrate (about 50%) occurs for the most part as polyhexose. Glucose is the predominant sugar residue, but galactose and probably mannose are also present in the polysaccharide.

5. The presence of chitin (about 5.5%) was confirmed, but it was only isolated associated with carbohydrate.

6. There is no indication of the presence of lignin.

I wish to thank the Agricultural Research Council for a grant. I am indebted to Dr P. W. Brian of the Butterwick Research Laboratories of Imperial Chemical Industries for generous supplies of mycelium, and to Mr P. J. Curtis who supervised its cultivation.

REFERENCES

- Abraham E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A. & Florey, H. W. (1941). *Lancet*, **241**, 177.
- Akasi, S. (1939). *J. Biochem., Tokyo*, **29**, 21.
- Black, M. M. & Schwartz, H. M. (1950). *Analyst*, **75**, 185.
- Blix, G. (1948). *Acta chem. scand.* **2**, 467.
- Bohonos, N., Woolley, D. W. & Peterson, W. H. (1942). *Arch. Biochem.* **1**, 319.
- Brian, P. W. (1949). *Ann. Bot., Lond.*, **13**, 59.
- Brown, F. (1950). *Biochem. J.* **47**, 598.
- Conway, E. J. (1947). *Microdiffusion Analysis and Volumetric Error*, 2nd ed. London: Crosby Lockwood.
- Elson, L. A. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
- Flood, A. E., Hirst, E. L. & Jones, J. K. N. (1948). *J. chem. Soc.* p. 1679.
- Foster, J. W. (1949). *Chemical Activities of Fungi*. New York: Academic Press.
- Gorgica, H. J., Peterson, W. H. & Steenback, H. (1934). *Biochem. J.* **28**, 504.
- Holden, M., Pirie, N. W. & Tracey, M. V. (1950). *Biochem. J.* **47**, 399.
- Hough, L., Jones, J. K. N. & Wadman, W. H. (1950). *J. chem. Soc.* p. 1702.
- Immers, J. & Vasseur, E. (1950). *Nature, Lond.*, **165**, 898.
- Irving, J. C. (1909). *J. chem. Soc.* p. 564.
- Krüger, D. & Tschirch, E. (1930). *Ber. dtsh. chem. Ges.* **63**, 826.
- Kuttner, T. & Lichtenstein, L. (1932). *J. biol. chem.* **95**, 661.
- Markham, R. (1942). *Biochem. J.* **36**, 790.
- Nelson, N. (1944). *J. biol. chem.* **153**, 375.
- Norman, A. G. & Peterson, W. H. (1932). *Biochem. J.* **26**, 1946.
- Norman, A. G., Peterson, W. H. & Houtz, R. C. (1932). *Biochem. J.* **26**, 1934.
- Olmsted, W. A., Whitaker, W. M. & Duden, C. W. (1929). *J. biol. Chem.* **85**, 109.
- Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
- Phillips, M. (1938). *J. Ass. off. agric. Chem., Wash.*, **21**, 678.
- Pinck, L. A. & Allison, F. E. (1944). *Soil Sci.* **57**, 155.
- Pirie, N. W. (1936). *Brit. J. exp. Path.* **17**, 269.
- Porges, N. (1932). *Bot. Gaz.* **94**, 197.
- Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
- Schulz, G. (1937). *Planta*, **27**, 196.
- Takata, R. (1929). *J. Soc. chem. Ind. Japan*, **32**, 245 B.
- Thom, C. & Phillips, M. (1932). *J. Wash. Acad. Sci.* **22**, 230.
- Tracey, M. V. (1951). *Biochem. J.* **49**, xx.
- Van Slyke, D. D., Dillon, R. T., Macfadyen, D. A. & Hamilton, P. (1941). *J. biol. Chem.* **141**, 671.
- Waksman, S. A. (1938). *Humus*. 2nd ed. London: Baillière, Tindall and Cox.

The Component Acids of the Fats of some Indian Fresh-water Fish

BY S. P. PATHAK AND C. V. AGARWAL

Department of Industrial Chemistry, Benares Hindu University

(Received 3 September 1951)

The fats studied were from the body, viscera and liver of bhakur fish (*Catla buehanani*) and from the liver of pahuna fish (*Wallago attu*). These fish were from the River Ganges at Benares.

METHODS

The separated tissues (body, viscera or liver) were cut up into small pieces and extracted exhaustively with hot acetone. The extracted material was then taken up in light petroleum (b.p. 40–60°), the watery portion separated and the lipids recovered and dried at 100° under reduced pressure. The body and liver tissues were also treated by alkali digestion as recommended by Rapson, Schwartz & Van Rensburg (1943), but little fat could be extracted by this treatment. The fats were kept in 10 vol. of acetone at 0° to separate the phosphatides, if present, from the glycerides.

The fats were hydrolysed, and the mixed fatty acids recovered were resolved into groups of simpler mixtures of acids by the lithium salt-acetone (Tsujimoto, 1920; Tsujimoto & Kimura, 1923) and lead salt-ethanol methods. Each group of the acids was separately converted into methyl

esters, taking precautions recommended by Bjarnason & Meara (1944), and fractionated through an electrically heated and packed column. From the iodine values and equivalents of the ester fractions the composition of the latter were calculated by the method of Hilditch (1947). The mean unsaturation, expressed by the fractional number of hydrogen atoms short of saturation, e.g. –2.0 (monoethenoid), was determined as usual by interpolation or extrapolation from the respective ester fractions in each group from which the mean equivalent of each of the homologous ester groups (C₁₆, C₁₈, C₂₀, C₂₂ and C₂₄) follows. From the composition of each of the separated groups of fatty acids, that of the original fat was obtained.

RESULTS

The lipids and phosphatide contents of the tissues and the iodine values of the fats are given in Table 1.

Bhakur fats. The mixed fatty acids obtained by the hydrolysis of fats, as described earlier, from body (159.6 g.), viscera (202.8 g.) and liver (29.3 g.) were