Further Study of the Lipids from *Pithomyces chartarum* (*Sporidesmium bakeri*) and Related Fungi

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A study of the lipids of *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis (which has replaced the name *Sporidesmium bakeri* Syd. used previously) has shown that their fatty acid composition differs from that of other fungi hitherto examined in containing large quantities of both linoleic acid and palmitic acid (Hartman, Hawke, Morice & Shorland, 1960).

The main object of the present work has been to determine whether the fatty acid composition of the lipids of *P. chartarum* is peculiar to that species or common to other members of the same or related families. For this purpose two further samples of *P. chartarum* as well as a sample of another dark-coloured fungus, *Stemphylium dendriticum* from the same family, Dematiaceae, and a sample of a hyaline or bright-coloured fungus, *Cylindrocarpon radicumicola* from the related Tuberculariaceae family, have been investigated. The spores from *P. chartarum* and a sample of the medium on which all the fungi were grown have also been examined.

As Weenink (1961) and Shorland (1961) have shown that some plant materials with relatively small amounts of lipids contain in the acetone-soluble portion only small amounts of triglycerides, the nature of the non-phospholipid fractions of

fungus lipids has been examined by isolation and determination of glycerol, as well as by the estimation of the sterol content.

EXPERIMENTAL

The fungi were grown on a potato and carrot infusion and collected as described by Hartman *et al.* (1960). The spores of *P. chartarum*, grown on rye grain at 24° for 21 days, were obtained in a high degree of purity by passing the crude material through a series of fine sieves until all coarser extraneous matter was eliminated. Extraction of the fungal mats was simplified by omitting the partition of lipids between methanol and light petroleum, b.p. 40–60° (the scheme followed in the extraction of the hepatotoxin responsible for 'facial eczema'). The extraction scheme is shown in Fig. 1. All extractions were carried out by refluxing for 15 min. This scheme applied to all the specimens, except that the spores, being practically dry, did not require dehydration with acetone. An amount of nutrient medium approximately equal to that used for growing the various fungi was evaporated under vacuum almost to dryness and then extracted similarly.

The lipids from extract A, except the spores and the medium, were dialysed against light petroleum in a rubber membrane (see Hartman *et al.* 1960) to give 'glyceride' and 'phospholipid' fractions. The 'glycerides' were titrated in ethanol with aqueous 0.1N-potassium hydroxide in the presence of phenolphthalein, and, after addition of aqueous

Fig. 1. Extraction scheme.

10% potassium carbonate, extracted with ether. The ether extract, washed free of alkali with water, yielded the neutral 'glycerides'. Acidification of the alkaline layer and extraction with ether gave the free fatty acids. These acids as well as those obtained after the usual saponification and removal of unsaponifiable matter from the neutral 'glycerides', the 'phospholipids' and extract *B* were converted into methyl esters (Hilditch, 1956). The composition of the esters was determined on a Pye Argon Chromatograph (see Hartman *et al.* 1960).

Sterols in the neutral 'glyceride' fractions were determined as ergosterol by the method of Zak, Luz & Fisher (1957) as modified by Gerson (1960), with ergosterol as a standard in place of cholesterol, and measuring the extinction at 455 m μ , which is the wavelength of maximum absorption for the ergosterol derivative produced by the action of the ferric chloride-acetic acid-sulphuric acid reagent. Free sterols were determined in the neutral 'glycerides', and total sterols in the unsaponifiable matter from these fractions.

To identify the glycerol in the 'glyceride' portion of the lipids from *P. chartarum*, a sample of neutral 'glycerides' was saponified, the solution acidified with dilute sulphuric acid, and the lipids were extracted with ether. The neutralized aqueous layer was evaporated *in vacuo* in a rotary evaporator, the residue extracted three times with small portions of methanol, and the solvent evaporated. The methanolic extract was taken up in dry pyridine and filtered. After removal of the pyridine and leaving in a vacuum desiccator for 3 days the remaining syrupy liquid was identified as glycerol by preparing its tri-*p*-nitrobenzoyl derivative and determining its m.p. The purity of the glycerol thus obtained was determined by the potassium periodate method (Hartman, 1953) by titrating the formic acid produced.

The content of glycerol in the 'glyceride' portion was established by the potassium periodate method with the neutralized aqueous layer obtained after saponification of a sample of neutral 'glycerides' and removal of lipids. In other samples of the same neutral 'glycerides' acetyl values and the amounts of unsaponifiable matter, fatty acids and α -monoglycerides were determined by standard methods.

RESULTS AND DISCUSSION

Yield of lipids. The weights of lipids obtained, their percentage based on total dry matter and the percentage of various fractions are given in Table 1. As the fatty acids obtained from the nutrient medium were only 1% of those from *P. chartarum* 1,

grown on a similar amount of medium, it is evident that the fatty acids of the fungal mats were synthesized by the fungi themselves. The yields of lipids from *P. chartarum* were similar to those reported in our previous investigation (4–6%). *S. dendriticum* contained less lipids than *P. chartarum*, and *C. radiculicola* more. By the addition of 1% of conc. hydrochloric acid to the extraction solvents only a very small amount of material was obtained from *P. chartarum*. This was insoluble in light petroleum and discarded. In contrast *S. dendriticum* yielded under these conditions 0.168 g. of lipid material soluble in light petroleum, and *C. radiculicola* 0.786 g.

Component fatty acids. The component fatty acids of the various fractions of extract *A* and of extract *B*, expressed as molar percentages, are shown in Table 2. In Table 3 are listed the component fatty acids of the total lipids of *P. chartarum* spores, *S. dendriticum* and *C. radiculicola*. The total fatty acids of the fungal mats of *P. chartarum*, except for two additional components (mono-unsaturated and saturated C₂₀ acids) are similar to those already reported (Hartman *et al.* 1960). Extract *B* and the various fractions of extract *A* of the three fungi do not vary greatly in fatty acid composition from the total; the only exception is the 'phospholipid' fraction of *C. radiculicola*, which contains 49% of linoleic acid, a percentage much higher than in any of the other fractions of *C. radiculicola*. In all the fractions of both *P. chartarum* and *S. dendriticum* linoleic acid is the most abundant acid, 36–69%, but in *C. radiculicola*, apart from the 'phospholipid' fraction, its amount (22–28%) is slightly exceeded by that of oleic acid (27–31%). The 'phospholipids' of all the fungi contain the highest percentage of linoleic acid, whereas the neutral 'glycerides' of *P. chartarum* and *S. dendriticum* are consistently richer in oleic acid than the other fractions. The extract *B* lipids of *S. dendriticum* and *C. radiculicola* resemble more the neutral 'glycerides' than the other fractions of extract *A*.

In total fatty acid composition the spores of *P. chartarum* are similar to the fungal mats. *S. dendriticum*, belonging to the same family as

Table 1. *Percentage of various fractions of fungal lipids*

	Composition of extract <i>A</i> (%)			Total lipids			
	Free fatty acids	Neutral 'glycerides'	'Phospho-lipids'	Extract <i>A</i> (%)	Extract <i>B</i> (%)	Weight (g.)	% of dry matter
<i>P. chartarum</i> 1	21.1	64.5	14.4	100.0	Trace	1.745	4.58
<i>P. chartarum</i> 2	19.8	54.1	26.1	100.0	Trace	4.696	4.07
<i>P. chartarum</i> spores		Not separated		100.0	Trace	0.216	1.39
<i>S. dendriticum</i>	15.1	65.2	19.7	86.7	13.3	1.268	2.40
<i>C. radiculicola</i>	11.4	64.8	23.9	78.0	22.0	3.572	7.50
Nutrient medium		Not separated		100.0	0	0.096	0.07

Table 2. Component fatty acids of lipid fractions from *Pithomyces chartarum*, *Stemphylium dendriticum* and *Cylindrocarpum radiclecola* expressed as molar percentages

Fatty acids* ...	11:0	12:0	13:0	14:0	15:0 br.†	15:0	16:1	16:0	17:0 br.†	17:0	18:3	18:2	18:1	18:0	20:1
Extract A															
Free fatty acids															
<i>P. chartarum</i> 1	0.4	1.6	—	1.6	—	1.0	1.0	23.2	1.6	—	4.2	48.5	11.6	5.3	—
<i>P. chartarum</i> 2	Trace	Trace	Trace	1.3	—	0.3	1.1	29.3	—	—	1.7	55.7	5.6	5.0	—
<i>S. dendriticum</i>	Trace	Trace	Trace	0.5	—	—	0.5	13.8	—	—	10.4	57.4	11.3	6.1	Trace
<i>C. radiclecola</i>	1.3	0.4	—	0.6	—	—	1.7	32.8	—	—	4.4	22.3	27.3	9.2	—
Neutral 'glycerides'															
<i>P. chartarum</i> 1	—	—	—	2.6	—	0.4	0.4	33.9	—	—	0.9	35.6	17.6	8.6	—
<i>P. chartarum</i> 2	0.2	—	—	0.5	—	0.3	0.8	26.2	—	—	2.1	41.6	20.1	8.2	—
<i>S. dendriticum</i>	—	0.3	—	0.7	—	0.1	2.0	21.9	—	—	2.7	47.7	21.7	2.9	—
<i>C. radiclecola</i>	—	—	—	0.3	—	—	0.6	23.5	—	—	10.9	27.9	28.6	8.2	—
'Phospholipids'															
<i>P. chartarum</i> 1	Trace	0.8	—	0.4	0.8	—	2.3	15.4	0.8	—	6.1	56.2	6.9	5.4	4.9†
<i>P. chartarum</i> 2	Trace	0.9	0.2	0.5	0.5	Trace	0.7	19.0	2.4	0.9	3.8	62.1	4.7	1.9	2.4
<i>S. dendriticum</i>	Trace	0.1	2.1§	0.9	—	—	1.1	12.1	—	0.7	2.0	69.3	7.6	2.6	1.5
<i>C. radiclecola</i>	—	1.0	—	0.4	—	1.1	0.7	16.8	0.9	1.7	13.3	49.3	11.3	3.1	0.4
Extract B															
<i>S. dendriticum</i>	—	—	—	0.6	—	—	1.8	17.8	—	—	16.7	42.8	16.7	3.6	—
<i>C. radiclecola</i>	—	—	—	0.2	—	—	0.3	22.4	—	—	8.9	28.3	31.0	8.9	—

* Designated by the number of carbon atoms followed by the number of double bonds.

† Branched-chain.

‡ Contains 0.7 of C₂₀.§ Contains 0.7 of C₁₃ branched-chain.|| Contains 0.4 of C₁₉ monounsaturated.

P. chartarum, the Dematiaceae, resembles it in containing a large amount of linoleic acid, 52 %, and comparable amounts of palmitic, oleic and stearic acids. *C. radicola* belonging to the same order, Moniliales, but to a different family, the Tuberculariaceae, has a different fatty acid composition from the two members of the Dematiaceae. Its content of linoleic acid is much lower, 29 %, and of oleic acid higher, 27 %. At the same time it differs from *Penicillium spinulosum* (Shimi, Singh & Walker, 1959), *Penicillium lilacinum* (Singh, Shah & Walker, 1956a), *Penicillium flavocinereum* (Singh, Shah & Walker, 1956b), *Penicillium javanicum* (Ward & Jamieson, 1934) and *Aspergillus nidulans* (Singh & Walker, 1956), all members of the Moniliales but of the family Moniliaceae, which contain a greater quantity of oleic acid, 35–43 % (w/w), than of any other acid.

Some individual fatty acids such as chaulmoogric, petroselinic and erucic acid are characteristic of seed fats of certain families, the Flacourtiaceae, the Umbelliferae and the Cruciferae respectively (Hilditch, 1956). The fatty acids present in the fungal lipids now investigated are those commonly found in seed and leaf lipids, and it is not possible to use their presence as a guide in botanical classification. On the other hand, as each of the three families of fungi mentioned before has a distinctive fatty acid composition, it seems possible that fatty acid composition of fungi may have some taxonomic significance, as it has in the seed fats of the Palmae. However, in view of the

variety of factors involved, any broad generalizations concerning the taxonomy of fungi based on fatty acid composition do not yet seem justified. For example the amount of linolenic acid in the lipids of *Neurospora crassa* (Todd, Stone, Hechter & Nussbaum, 1957) may depend on whether the mycelia are grown by surface or submerged culture techniques.

Sterols. The amounts of total and free sterols appear in Table 4 and show close agreement between the two batches of *P. chartarum*. *S. dendriticum* contains a higher percentage of free and total sterols than the other two fungi. On the other hand, both *S. dendriticum* and *C. radicola* contain less combined sterol than *P. chartarum*. Although the sterols have been determined as ergosterol, which is a well-known constituent of fungal lipids (Fieser & Fieser, 1949; Bernhard & Albrecht, 1948), it is probable that there are other sterols present as well.

Identification and content of glycerol in neutral 'glycerides'. A light-petroleum extract of *P. chartarum* spores (20–400 g.) (kindly supplied by Dr E. P. White of the Ruakura Animal Research Station) was separated into 15.885 g. of neutral lipids and 4.463 g. of free fatty acids. The neutral lipids yielded on dialysis 13.752 g. of 'glycerides', from which 0.927 g. of glycerol of 96.4 % purity as determined by the periodate method was obtained. From 0.055 g. of this product and 0.6 g. of freshly prepared *p*-nitrobenzoyl chloride, 0.3 g. of a derivative was obtained, m.p. 192–193°, which did

Table 3. *Component fatty acids of the total lipids from Pithomyces chartarum spores, Stemphylium dendriticum and Cylindrocarpon radicola expressed as molar percentages*

Fatty acids	<i>P. chartarum</i> spores	<i>S. dendriticum</i>	<i>C. radicola</i>
<i>n</i> -C ₁₁	0.6	Trace	0.2
<i>n</i> -C ₁₂	—	0.2	0.1
Branched-chain C ₁₃	—	0.1	—
<i>n</i> -C ₁₃	—	0.2	—
<i>n</i> -C ₁₄	0.8	0.6	0.3
<i>n</i> -C ₁₅	Trace	Trace	0.1
Palmitoleic	1.3	1.6	0.7
<i>n</i> -C ₁₆	21.6	18.6	23.8
Branched-chain C ₁₇	—	—	0.1
<i>n</i> -C ₁₇	—	0.1	0.2
Linolenic	—	6.2	9.8
Linoleic	53.4	51.5	29.3
Oleic	15.9	17.2	27.4
<i>n</i> -C ₁₈	6.4	3.6	8.0
Monounsaturated C ₁₉	—	Trace	—
Monounsaturated C ₂₀	—	0.1	Trace

Table 4. *Percentage of total and free sterols in neutral 'glyceride' fractions*

	<i>P. chartarum</i> 1	<i>P. chartarum</i> 2	<i>S. dendriticum</i>	<i>C. radicola</i>
Total sterol	8.3	8.4	15.6	9.6
Free sterol	5.4	5.7	14.3	8.4

not change after recrystallization from acetone and did not depress the m.p. of authentic glycerol tri-*p*-nitrobenzoate. Jacquemain & Muscovits (1936) give m.p. 192°.

Neutral 'glycerides' from *P. chartarum* 2 (2.007 g.) yielded 0.127 g. of glycerol as determined by the periodate method in the aqueous layer obtained after saponification and extraction of lipids. The amount of the recovered fatty acids with a mean molecular weight of 274.0 was 1.333 g. and the content of α -monoglycerides in the original material 1.5 %. Thus about 83 % of the total fatty acids was apparently present in the form of triglycerides. It would seem that the remainder of the fatty acids was combined with unidentified alcohols, which formed a considerable portion of unsaponifiable matter as evidenced by its acetyl value of 111.5, and to a lesser extent (approximately 3 % of the total acids) with sterols.

SUMMARY

1. The fatty acid composition (molar percentages) of the lipids from *Stemphylium dendriticum*, family Dematiaceae, consisted of 52 % of linoleic, 19 % of palmitic and 17 % of oleic acid as major components, thereby resembling that of the fungal mats and spores of *Pithomyces chartarum*, another member of the same family. In contrast, the fatty acid composition of *Cylindrocarpon radiculicola*, from the related Tuberculariaceae family, resembled more that of other fungi hitherto examined in containing 29 % of linoleic, 24 % of palmitic, 27 % of oleic and 10 % of linolenic acid.

2. The amounts of total sterols in the 'glyceride' fraction of *Pithomyces chartarum* and *Cylindrocarpon radiculicola* were found to be from 8.3 to 9.6 % and of free sterols from 5.4 to 8.4 %, whereas the corre-

sponding amounts of *Stemphylium dendriticum* were 15.6 and 14.3 %.

3. Glycerol has been isolated from the 'glycerides' of *Pithomyces chartarum*, their saponifiable fraction consisting predominantly of triglycerides.

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Further Experiments on the Potassium Uptake by *Alcaligenes faecalis*

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Krebs, Whittam & Hems (1957) have shown that *Alcaligenes faecalis* readily loses its intracellular potassium on washing if the potassium concentration of the washing medium is low, and it recovers the lost ion when placed in dilute solutions of potassium salts. The recovery of potassium was

found to be due partly to active transport depending on respiration, but even without a supply of energy considerable amounts of potassium were taken up against concentration gradients. Measurements of the simultaneous movements of K^+ , Na^+ and Cl^- ions failed to explain how electroneutrality