Phytochemistry, 1976, Vol. 15, pp. 971–975. Pergamon Press. Printed in England.

LIPID COMPOSITION OF *SPORENDONEMA EPIZOUM*

A. LOPEZ and J. BURGOS
Laboratory of Biochemistry and Food Technology, Facultad de Veterinaria, León, Spain

(*Revised received* 27 *January* 1976)

Key Word Index—*Sporendonema epizoum*; Moniliales; fungi imperfecti; growth phases; lipid composition; fatty acids.

Abstract—Triglycerides, free fatty acids, free and esterified ergosterol, Q_9, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, lysophosphatidylethanolamine, and three different acylglycoses were identified in the soluble lipids of *Sporendonema epizoum* mycelium. The same compounds as well as a sterol glycoside were also found in conidia. The mycelium is richer than the conidia in phospholipids, Q_9 and free and esterified ergosterol but contains less glycolipids. The most abundant fatty acid in all non-polar fractions is $C_{18:2}$. The prevalent fatty acid of the phospholipids is $C_{18:1}$, except for conidial phosphatidylethanolamine and mycelial lysophosphatidylethanolamine.

INTRODUCTION

The lipid composition of fungi has attracted less attention than that of bacteria and other organisms, although it is well known that many fungi are able to accumulate large amounts of cellular lipids. A detailed knowledge of the composition in different growth phases and under different cultural conditions (since it is known that the lipid composition varies with age and temperature [1] and supply of nutrients [2]) would contribute to a better understanding of the role of this type of compound in fungi and could be an aid to taxonomy. Early attempts to find a relationship between lipid composition and fungal taxonomy failed [3], but this was probably due to lack of detailed information. Several investigators have reported the fatty acid composition of some fungal total lipids and less frequently that of some lipid fractions [4–7] but little information is available on the distribution of fatty acids among individual fungal lipid components and their possible changes with growth phases.

The work reported here is part of a general study of the biochemistry of *Sporendonema epizoum* [8] (Synonym: *Hemispora stellata* [9]). This is a highly aerobic fungus often found in salted fish where it produces a kind of spoilage known as "dun"; it belongs to the order Moniliales, family Dematiaceae. Some strains are able to grow in the absence of NaCl but most of them require concentrations greater than 10%. Some reports on its cytochrome system [10] and the relationship between its Q content and respiration [11] have already been published. In this paper we have identified and analysed the various compounds which make up the cellular lipids of conidia and young mycelium of this fungus, as well as the fatty acid components of the various components identified.

RESULTS AND DISCUSSION

As shown in Table 1, the lipid content is slightly higher in young mycelium than in conidia; about 80% consists of soluble lipid, 80% of which are nonpolar components, the rest (polar lipids) being glyco and phospholipids. The polar lipids are rather rich in glycolipids, especially in conidia where they are about 3 times more abundant than phospholipids.

Nonpolar lipids. The nonpolar fraction of soluble lipids displayed 8 spots on TLC. Spots 1 to 5 (numbered in decreasing order of polarity) contained ergosterol esters, triglycerides, Q_9, free fatty acids, and ergosterol respectively. When the material of spots 1, 3 and 5 were extracted from the Si gel after TLC the weight of the recovered samples greatly exceeded those determined spectrophotometrically. Lipids accompanying these compounds were not identified, but since in the TLC system used, squalene and other hydrocarbons migrated together with the ergosterol esters, it was deduced that spot 1 must have contained hydrocarbons as well as ergosterol (and possibly other sterols) esters. Spot 6, 7 and 8 gave colours ranging from green to blue with

Table 1. Lipid yield from conidia* and young mycelium† of *Sporendonema epizoum* and chromatographic behaviour of the soluble lipids on silicic acid columns

Growth phase	Soluble lipids: % dry wt	% eluted from silicic acid columns with: $CuCl_3$‡	Me_2CO¶	MeOH§	Bound lipids (% dry/wt)
Conidia	3.0	81.8	13.0	5.2	0.7
Mycelium	3.7	84.7	8.1	7.2	1.0

* Average of three experiments performed with 30, 26 and 16 g (dry wt). † Average of three experiments performed with 30, 35 and 27 g (dry wt). ‡ This material gave negative reaction when submitted to phosphorus, nitrogen and carbohydrate analysis (Nonpolar lipids). ¶ Negative phosphorus and nitrogen tests; positive anthrone reaction (glycolipids). § Negative anthrone reaction; positive nitrogen and phosphorus tests (phospholipids).

Table 2. Nonpolar components of the soluble lipids of conidia and mycelium of *Sporendonema epizoum*

Component	Conidia		Mycelium	
	% soluble lipids	% dry wt	% soluble lipids	% dry wt
Ergosterol esters	0.2	0.005	0.6	0.02
Other sterol esters plus hydrocarbons*	2.1	0.06	2.5	0.09
Triglycerides	3.1	0.09	4.3	0.2
Ubiquinone	0.04	0.001	0.3	0.01
Ubiquinone accompanying lipids†	5.2	0.2	6.7	0.3
Free fatty acids	28.1	0.8	28.9	1.1
Ergosterol	0.5	0.01	3.9	0.1
Other sterol and sterol-accompanying lipids‡	31.9	1.0	35.3	1.3

* Wt of the material of spot 1 in TLC in excess of the true ergosterol esters spectrophotometrically determined. † Wt of the spot 3 material in excess of the true ubiquinone content spectrophotometrically determined. ‡ The sum of the material of spots 6, 7 and 8 plus the wt of spot 5 in excess of the true ergosterol content spectrophotometrically determined.

H_2SO_4; the UV spectra of all these materials after preparative-TLC purification had maxima at 272 and 282 nm, but some lacked the 292 nm and others the 262 nm maxima of ergosterol and are referred to as non-identified sterols. Table 2 shows the conidial and mycelial contents of all these nonpolar compounds.

In contrast with what has been reported by other authors [3, 12, 13] for the major classes of fungi the most abundant identified component of the nonpolar lipids of *S. epizoum* was free fatty acids, the amounts of which were 7 to 9 times more than those of triglycerides in both growth phases. This finding cannot be due to the action of lipases on triglycerides during extraction, since no mono- or di-glycerides were detected among soluble lipids.

Ergosterol, although the only identified sterol, does not seem to be the most abundant one in this fungus. It should be noted that ergosterol is frequently accompanied in fungi imperfecti by two or three other 4-desmethylsterols [14]. Both, free and esterified ergosterol were much more abundant in mycelium than in conidia, the free form being in both growth phases clearly predominant. The prevalent fatty acid in all subfractions of nonpolar lipids is $C_{18:2}$ (Table 3), except for the sterol esters of conidia. The other major fatty acids of all components of the nonpolar lipids are C_{16} and $C_{18:1}$. The abundance of $C_{18:2}$ in the nonpolar lipids increases considerably in the mycelium where it represents more than 60% of all fatty acids; this increase is parallel to a decrease of C_{16} and $C_{18:3}$, (which is not present in mycelium lipids) and $C_{18:1}$. The decrease of $C_{18:1}$ in mycelium disagrees with what has been reported by Van Etten and Gottlieb [15] in *Penicillium atrovenetum*. Also the overall degree of unsaturation of nonpolar lipids is higher in mycelium than in conidia, in contrast with what has been reported for other fungi [15–18].

Polar lipids. TLC of the glycolipids fraction of mycelium revealed the presence of three spots tentatively characterized as acylglycoses. The glycolipids fraction of conidia showed the three components observed in mycelium with another one characterized as sterolglycoside, a type of compound rather uncommon in microbial lipids. The presence of acylglycoses, although more frequent in bacteria, is not an unusual feature of fungi where fatty acids attached to a carbohydrate via a glycosidic linkage [19, 20] and acylated polyols [21, 22] have been detected.

The phospholipid fraction of both growth phases consist of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine (all of them frequently found in most fungi) [23] and lysophosphatidylethanolamine (which has been occasionally reported in other fungal species) [12].

Few detailed studies have been made on the quantitative distribution of phospholipids in fungi [23]. From the work of Batia *et al.* [12], phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol appear to be the most abundant in the mycelium of the major classes of fungi. Comparisons between the phospholipid distribution of spores and mycelium are even more scarce and the results rather conflicting [24]. In the mycelium of *S. epizoum* phosphatidylethanolamine and phosphatidylcholine are the most abundant (Table 4) and lysophosphatidylethanolamine is the prevalent phospholipid in conidia where the other three are in *ca* equal proportions. Their fatty acid composition is shown in Table 5. In all conidial phospholipids the dominant fatty acid is $C_{18:1}$, except for phosphatidylethanolamine in which C_{16}, $C_{18:1}$ and $C_{18:2}$ are in *ca* equal proportions. This dominance of $C_{18:1}$ in the phospholipid is less pronounced in mycelium, in one of whose phospholipids, lysophosphatidylethanolamine, $C_{18:2}$ is much more abundant than $C_{18:1}$.

It is worth noting the following points: phosphatidylethanolamine is the only phospholipid containing $C_{16:2}$, which is present in all nonpolar subfractions: phosphatidylcholine is, in both growth phases, about twice as rich in C_{16} as any other phospholipid; no phospholipid of either conidia or mycelium contain any fatty acid of more than two double bonds and the percentage of $C_{18:2}$ is very low in all phospholipids.

Table 3. Fatty acid composition of nonpolar soluble lipids from conidia and young mycelium of *Sporendonema epizoum* (wt %)

Fatty acid	Conidia			Mycelium		
	Free fatty acid (%)	Triglycerides (%)	Sterol esters (%)	Free fatty acid (%)	Triglycerides (%)	Sterol esters (%)
C_8	—	0.01	1.2	—	—	1.5
C_{10}	—	0.02	0.12	—	—	0.2
C_{12}	0.2	0.03	5.7	—	—	1.3
C_{14}	0.6	0.02	3.5	0.5	0.4	1.1
C_{16}	25.6	16.8	32.1	17.8	18.2	10.8
$C_{16:1}$	0.9	0.02	1.4	0.9	0.3	1.0
$C_{16:2}$	1.2	0.3	1.4	0.04	0.3	0.9
C_{18}	1.8	1.2	7.5	3.5	2.3	5.8
$C_{18:1}$	23.8	19.7	11.8	11.5	10.1	14.8
$C_{18:2}$	45.2	54.8	24.5	65.1	68.2	61.1
$C_{18:3}$	0.5	4.2	—	—	—	—
C_{20}	—	—	11.3	—	—	—

Table 4. Phospholipids of conidia and mycelium of *Sporendonema epizoum*

Growth phase	% of total phospholipids			
	Phosphatidyl-ethanolamine	Phosphatidyl-choline	Lysophosphatidyl-ethanolamine	Phosphati-dylserine
Conidia	20.9	24.6	30.6	23.7
Mycelium	34.0	35.3	16.1	14.6

The overall degree of unsaturation is smaller in the phospholipids than in the nonpolar fraction, particularly in conidia; mycelium phosphatidylethanolamine and lysophosphatidylethanolamine are the more unsaturated phospholipids, but their degree of unsaturation is lower than that of triglycerides or free fatty acids; this disagrees with what has been reported in other fungi by White and Powell [5].

Considered as whole, in the soluble lipids of conidia and mycelium of *S. epizoum* the unsaturated fatty acids are dominant and represent *ca* 60% of the total fatty acids. This abundance of unsaturated fatty acids seems to be a general characteristic of fungal lipids [4, 24, 25].

In the soluble lipids of *S. epizoum* no fatty acids of more than 18 C have been detected (except for C_{20} in conidia sterol esters) nor fatty acids with an odd C number, which are known to be present in other fungi of the same family (Dematiaceae) [4, 26] and in other Moniliales [6]. No branched acids, which have been found in small proportions in other Dermateaceae [4, 26], or hydroxy fatty acids have been detected.

Fatty acids shorter than C_{14} have not been usually reported in fungi but in the sterol ester fraction, however, of both growth phases of *S. epizoum* significant amounts of shorter chain fatty acids (down to C_8) occur.

Bound lipids. Hydrocarbons, ubiquinone, mono and diglycerides, ethyl esters of fatty acids, ergosterol and other sterols were the only compounds identified. Ergosterol was not detected in conidia. In both growth phases, it was found that the contribution of each kind of lipids (estimated by wt) to the total bound lipids was as follows: hydrocarbons, *ca* 20%; ethyl esters of fatty acids *ca* 5%; total sterols *ca* 25–30%; mono and diglycerides 45–50%. Q_9 whose estimation under these conditions has already proved to be reliable [11] represented 0.0006% of the dry wt in conidia and 0.0057% in mycelium; this means that *ca* 30% of the total Q in conidia and 37% in mycelium is not extracted with the soluble lipids.

Although identified by its chromatographic and spectrophotometric behaviour, no attempt was made to estimate ergosterol in the bound lipids of mycelium, since previous experiments had demonstrated [11] that under the extraction conditions great loss takes place.

The very high proportions of hydrocarbons among bound lipids is rather surprising, especially when compared to its scarcity in the soluble fraction. By treating samples of the soluble lipid under the experimental conditions used for the extraction of bound lipids we have proved that they are not artifacts of the extraction procedure. Mono- and di-glycerides, absent in the soluble fraction, are most probably the result of acid hydrolysis of phospholipids and triglycerides during extraction of bound lipids. The ethyl esters are obviously due to fatty acid ethylation when boiling with Me_2CO–EtOH–HCl.

Table 6 shows the fatty acid composition of the total bound lipids, which differs from that of the soluble lipids, in having unsaturated fatty acids of shorter chain length than C_{16}, odd C number homologues and in the presence of $C_{20:1}$ in mycelium.

Bound lipids show a similar trend toward higher unsaturation in mycelium already noted for the soluble fraction.

EXPERIMENTAL

S. epizoum was obtained from Centraalbureau voor Schimmelcultures (Baarn—Netherlands). This strain grows in malt agar and Vaisey medium [27] at NaCl concn varying between 0–20%, with an optimum concn of 7.5%. Conidia were obtained and purified as already described [28]. Young mycelia were produced by inoculating Vaisey medium containing 7.5% of NaCl, with aseptically obtained conidia. The cultures were grown aerobically for 48 hr at 25° in a reciprocal shaker

Table 5. Fatty acid composition of the individual phospholipids from conidia and mycelium of *Sporendonema epizoum*

Fatty acid	Conidia				Mycelium			
	Phospha-tidyl-choline (%)	Phospha-tidyl-serine (%)	Phospha-tidyl-ethanol-amine (%)	Lysophos-phatidyl-ethanol-amine (%)	Phospha-tidyl-choline (%)	Phospha-tidyl-serine (%)	Phospha-tidyl-ethanol-amine (%)	Lysophos-phatidyl-ethanol-amine (%)
C_8	—	—	—	—	0.2	—	—	—
C_{10}	0.3	1.1	10.8	2.0	4.8	4.8	—	1.0
C_{12}	0.9	2.2	3.0	—	5.7	5.3	—	1.5
C_{14}	1.7	2.2	3.6	2.0	5.2	5.3	—	4.0
C_{16}	10.3	13.3	24.0	12.0	6.4	9.5	21.3	7.7
$C_{16:1}$	3.4	—	0.4	0.4	3.8	2.6	1.4	1.1
$C_{16:2}$	—	—	2.1	—	—	—	1.4	—
C_{18}	6.0	1.1	6.0	0.4	5.0	2.3	7.5	4.9
$C_{18:1}$	65.6	72.7	22.0	72.0	65.0	62.0	35.4	35.0
$C_{18:2}$	11.9	6.6	27.5	12.0	3.4	7.6	33.3	44.5

Table 6. Fatty acids composition of bound lipids of conidia and mycelium of *Sporendonema epizoum*

Fatty acid	Conidia (%)	Mycelium (%)
C_8	0.3	1.5
C_{10}	1.6	7.5
C_{12}	0.2	1.9
C_{14}	1.8	0.8
$C_{14:1}$	0.02	0.6
$C_{14:2}$	0.02	1.2
C_{15}	0.06	—
C_{16}	10.0	16.5
$C_{16:1}$	1.3	1.2
$C_{16:2}$	0.5	0.2
C_{17}	0.5	3.8
C_{18}	12.1	2.3
$C_{18:1}$	65.7	35.9
$C_{18:2}$	4.6	5.6
$C_{18:3}$	0.6	19.8
$C_{20:1}$	—	3.7

(220 strokes/min). Mycelia were harvested by centrifugation at 2000 *g* for 5 min.

Lipid extraction. On the basis of the results of previous work [11] cell material was lyophilized and extracted × 3 with 10 vols of $CHCl_3$–MeOH (2:1) which contained 0.25% pyrogallol, for 3 hr at room temp with continuous stirring. The cell residue was extracted twice by refluxing for 2 hr with 5 vols of MeOH containing 0.25% pyrogallol. Lipid extracts were evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$–MeOH (2:1) and this soln washed according to the method of ref. [29]. Lipids extracted by this procedure were considered soluble or 'free lipids'. Bound lipids were obtained by extracting under reflux for 2 hr the cell residues from the previous extraction with 10 vol Me_2CO–EtOH–12N HCl (1:1:0.02). The cell residue was extracted × 3 and the extracts combined taken to dryness, dissolved in $CHCl_3$–MeOH (2:1) and back extracted and washed as previously described.

Fractionation of lipids. Silicic acid containing Celite (4/1) was packed as a slurry in $CHCl_3$–MeOH (98:2) into columns (2 × 25 cm). Lipid samples (40 mg/g of silicic acid) were applied in the same solvent and the column eluted successively with 300 ml $CHCl_3$, 200 ml Me_2CO and 200 ml MeOH. Aliquots of each fraction were analyzed for total N, P and carbohydrates.

Nonpolar lipids. Samples of the $CHCl_3$ eluates plus standards (squalene, triglycerides, mono- and di-glycerides, oleic and stearic acids, ergosterol and Q_9) were applied to Si gel TLC plates and developed in petrol–Et_2O–HOAc (80:20:1). Lipids were detected either with H_2SO_4, rhodamine 6G or I_2 vapour. Ergosterol and ergosterol esters were characterized from R_f values, the reddish colour they gave with H_2SO_4, and from the UV spectrum (maxima at 262, 271, 282, 293 nm in EtOH) after the sample had been purified by preparative-TLC. Q was visualized after spraying with leucomethylene blue [30]. When purified by preparative-TLC, the extracted material gave a UV spectrum with maxima at 272 nm in cyclohexane and 275 in EtOH. The 275 max in EtOH shifted to 290 nm when reduced with $NaBH_4$. The characterization of the free fatty acids fraction was based on the identity of its R_f with that of standard fatty acids and the intense fluorescence of the spot after spraying with rhodamine 6G.

Polar lipids. Aliquots of the lipids eluted from the silicic acid column with Me_2CO and MeOH were analyzed by TLC on Si gel G using $CHCl_3$–MeOH–H_2O (65:25:4). Lipids were visualized with the following spray reagents, 50% H_2SO_4, Schiff-periodate [31], molybdenum blue [32], ninhydrin and modified Dragendorff reagent [33]. The 4 spots visualized in the glycolipid fraction gave positive reactions with H_2SO_4, diphenylamine and Schiff reagents but were negative with ninhydrin. The characterization as acyl glycoses of three of the spots was based on their R_f, the above described staining behaviour and the lack of glycerol in their water soluble acid hydrolysis products. The identification as sterol glycoside of the fourth glycolipid was based on the red colour it gave with H_2SO_4, the lack of glycerol in its H_2O soluble acid hydrolysis products, and TLC behaviour and spectrum (two maxima about 270 and 280 nm) of its Et_2O soluble acid hydrolysis products. The different phospholipids were tentatively identified by comparison of their R_f with those of known standards and their staining behaviour. All gave positive reactions with H_2SO_4 and molybdenum blue reagents and negative reactions with Schiff reagent; one was positive with Dragendorff reagent and negative with ninhydrin (phosphatidylcholine) and the other three (phosphatidylethanolamine, lysophosphatidylethanolamine and phosphatidylserine) were positive with ninhydrin and negative with Dragendorff reagent. Their identities were confirmed by analysing the H_2O soluble acid hydrolysis products of samples purified by preparative-TLC. Individual lipids were separated by preparative-TLC. Lipids were visualized by spraying the outer edges of the plates with 0.5% I_2 in $CHCl_3$; nonpolar lipids were extracted from the Si gel by elution with Et_2O and glyco- and phospholipids as described in ref. [34].

Acid hydrolysis and PC of H_2O soluble products. Purified glycolipids and phospholipids were acid hydrolyzed as described in ref. [35]. The acid soluble fraction was dried under red pres, the residue dissolved in 3 ml of 95% EtOH and dried again to remove the last traces of HCl. The resulting residue was dissolved in H_2O and chromatographed together with known standards (choline, serine, ethanolamine and glycerol) on Whatman no. 1 paper, using *iso* PrOH–HOAc–H_2O (3:1:1) for phospholipids and *n*-BuOH–Py–H_2O (6:4:3) for glycolipids.

P, N and carbohydrate determination. Total N was determined by the method of ref. [36], P by the method of ref. [37] and carbohydrate with anthrone as described in ref. [36].

Quantitative estimation of the different components. Ubiquinone was estimated by the method of Crane [38]. Since we have already demonstrated that the Q homologue of *S. epizoum* is Q_9 [11] a $\Delta E^{1\%}_{1cm}$ (oxidized–reduced) at 275 nm of 158 was taken. Free ergosterol was determined by measuring its *A* at 282 nm in EtOH and using a $E^{1\%}_{1cm}$ of 298. The estimation of the total free fatty acids, esterified ergosterol and individual phospholipids was based on the average fatty acid MW of each fraction, deduced from their fatty acid composition, plus either the alkali titration of purified aliquots (free fatty acids), P determinations (phospholipids) or spectrophotometric measurements (ergosterol esters). Other components were estimated by determining the wt of the isolated material.

Fatty acid methylation and GLC of the methylesters. Free fatty acids were methylated with CH_2N_2 as described in ref. [39]; those of triglycerides and individual phospholipids by the method of ref. [40]; sterol esters were saponified by refluxing with 0.5 N KOH in EtOH for 30 min; the unsaponifiable material was extracted with Et_2O × 5; the aq fraction was acidified with 2 N HCl and fatty acids extracted with Et_2O × 5 and methylated as the free fatty acids. Fatty acids Me esters were purified by the microsublimation method of ref. [41] prior to GLC. Me esters were analysed by GLC using a chromatograph equipped with a dual FID. To identify the fatty acid constituents, polar and nonpolar columns were employed. The polar column (length 183 cm; i.d. 0.3 cm) was made of glass and packed with Chromosorb W coated with 10% by wt of DEGS. The operating conditions were: temp 50–180°, programmed 8°/min; N_2 flow, 35 ml/min. The nonpolar column, made also of glass and of equal length and i.d., was packed with Chromosorb W coated with 5% Apiezon L and was operated at 240° with a N_2 flow rate of 26 ml/min. To confirm the identification of fatty acids some samples were hydrogenated before they were chromatographed on both columns. Aliquots of the Me esters were submitted to TLC

on Si gel G as described by Morrison and Hay [42] to separate the esters of the normal and hydroxy fatty acids, if present. Fatty acid identification was based on R_f comparison with standards. Location of the double bond positions in unsaturated fatty acids was not attempted. For quantitative analysis, the area of the chromatogram under a peak for the Me ester of a given fatty acid was compared with that of a known amount of the corresponding standard.

REFERENCES

1. Bowman, R. and Mumma, R. D. (1967) *Biochem. Biophys. Acta* **144.** 501.
2. Garrido, J. M. and Walker, T. K. (1956) *J. Sci. Food Agric.* **7,** 233.
3. Jack, R. C. M. (1966) *J. Bacteriol.* **91.** 2101.
4. Hartman, L., Morice, I. M. and Shorland, F. B. (1962) *Biochem. J.* **82,** 76.
5. White, H. B. and Powell, S. S. (1966) *Biochem. Biophys. Acta* **116.** 388.
6. Stone, K. L. and Hemming, F. W. (1968) *Biochem. J.* **109.** 877.
7. Yusef, H. M., Threfall, D. R. and Goodwin, T. W. (1965) *Phytochemistry* **4.** 559.
8. Vuillemin, P. (1931) *Les champignons Parasites et les Mycoses de l'Homme* Lechevalier, Paris.
9. Ciferri, R. and Redaelli, P. (1934) *J. Trop. Med. Hyg.* **37.** 167.
10. Sala, F. J. and Burgos, J. (1937) *Arch. Mikrobiol.* **88.** 11.
11. Burgos, J., Sala, F. J. and Lopez, A. (1973) *Phytochemistry* **12.** 1201.
12. Bhatia, I. S., Raheja, R. K. and Chahal, D. S. (1972) *J. Sci. Food Agric.* **23.** 1197.
13. Leegwater, D. C., Youngs, C. G., Spencer, J. F. T. and Craig, B. M. (1962) *Can. J. Biochem. Physiol.* **40.** 847.
14. Weete, J. D. and Laseter, J. L. (1974) *Lipids* **9.** 575.
15. Van Etten, J. L. and Gottlieb, D. (1965) *J. Bacteriol.* **89,** 409.
16. Coots, R. H. (1962) *J. Lipid Res.* **3.** 84.
17. Leegwater, D. C., Youngs, C. G., Spencer, J. F. T. and Craig, M. B. (1962) *Can. J. Biochem. Physiol.* **40.** 847.
18. Bernard, K., Abisch, L. and Wagner, H. (1957) *Helv. Chim. Acta* **40.** 1291.
19. Gorin, P. A., Spencer, J. F. and Tulloch, A. P. (1961) *Can. J. Chem.* **39.** 846.
20. Tulloch, A. P., Spencer, J. F. T. and Deinema, M. H. (1968) *Can. J. Chem.* **46.** 345.
21. Fluharty, A. L. and O'Brien, J. S. (1969) *Biochemistry* **8.** 2627.
22. Brennan, P. J., Flynn, M. P. and Griffin, P. F. S. (1970) *FEBS Letters* **8.** 322.
23. Brennan, P. J., Griffin, P. F., Losel, D. M. and Tyrell, D. (1974), in *Progress in the Chemistry of Fats and other Lipids* (Holman, R. T. ed.), Vol. 14, Part 2, p. 51.
24. Jack, R. C. M. (1966) *J. Bacteriol.* **91.** 2101.
25. Singh, J. and Walker, T. K. (1956) *Biochem. J.* **62.** 286.
26. Hartman, L., Hawke, J. C., Morice, I. M. and Shorland, F. B. (1960) *Biochem. J.* **75.** 274.
27. Vaisey, E. B. (1954) *Fish. Res. Bd Can. Progs. Repts. Pacific. Coast. Stas.* **11** (6), 901.
28. Sala, F. J. and Burgos, J. (1972) *Appl. Microbiol.* **24,** 504.
29. Folch, J., Less, M. and Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226.** 497.
30. Goodwin, T. W. (1964) *Lab. Practice* **13.** 295.
31. Shaw, N. (1968) *Biochem. Biophys. Acta* **164.** 435.
32. Dittmer, J. C. and Lester, R. T. (1964) *J. Lipid Res.* **5.** 126.
33. LePage, M. (1964) *J. Chromatog.* **13.** 99.
34. White, D. C. and Frerman, F. E. (1967) *J. Bacteriol.* **94.** 1854.
35. Clark, J. M. (1964) , in *Experimental Biochemistry* (Clark, Jr. J. M. ed.), p. 61, Freeman, San Francisco.
36. Umbreit, W. W. and Burris, R. H. (1964) in *Manometric Techniques* (Umbreit, W. W., Burris, R. H. and Stauffer, J. F. eds.), p. 205, Burgess, Minnesota.
37. Fiske, C. H. and Subbarow, Y. (1925) *J. Biol. Chem.* **66.** 375.
38. Crane, F. L. (1959) *Plant. Physiol.* **34.** 128.
39. Schlenk, H. and Gellerman, J. L. (1960) *Analyt. Chem.* **32.** 1412.
40. Shehata, A. J. de Man, J. M. and Alexender, J. C. (1970) *Can. Inst. Food Technol.* **3.** 85.
41. Stoffel, W., Chu, F. and Ahrens, E. H. (1959) *Analyt. Chem.* **31.** 307.
42. Morrison, W. H. and Hay, J. D. (1970) *Biochem. Biophys. Acta* **202.** 460