The Cell-Envelope Glycolipids of Baker's Yeast

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Sphingolipids were found to dominate in the glycolipids from the cell envelope of baker's yeast. A relatively large quantity of ceramides was detected. Among the several complex phosphosphingolipids described, ceramide-(*P*-inositol)₂-mannose was the main component. About 55% of long-chain bases in sphingolipids consisted of C₁₈-phytosphingosine (4*D*-hydroxysphinganine). Other bases, found in decreasing concentrations, were C₂₀-phytosphingosine, C₂₀-dehydrophytosphingosine, C₁₈-dihydrosphingosine (sphinganine) and C₁₉-dihydrosphingosine. The presence of sterol glycosides, sulpholipids, cerebroside and acylglucoses was demonstrated.

The composition of glycolipids in baker's yeast (*Saccharomyces cerevisiae*) was examined with carefully isolated cell envelopes, which have been shown to contain fragments of plasma membrane as well as of the cell wall (Suomalainen *et al.*, 1967; Nurminen *et al.*, 1970). In the earlier studies, higher fatty acids (C₁₉–C₂₆) were observed to be distributed among acidic and neutral glycolipids (Nurminen & Suomalainen, 1971). The dominant fatty acids in a complex sphingolipid containing inositol, phosphorus and mannose were 2-hydroxy-C_{26:0} and C_{26:0}. In addition to the 2-hydroxy-C_{26:0} and other hydroxy fatty acids found, Hoshi *et al.* (1973) have isolated saturated unbranched 2,3-dihydroxy fatty acids with carbon numbers of 24–27 from the ceramide phosphate of baker's yeast.

The present work further investigated the sugar and sphingosine composition of the yeast envelope glycolipids. The glycolipids present in *S. cerevisiae* are known to be mainly sphingolipids, which are typical components of the plasma membrane in many cells. Wagner & Zofcsik (1966) isolated cerebroside and phosphosphingolipid (ceramide-*P*-inositol-mannose) from whole cells in *Candida utilis* and *S. cerevisiae*. They detected C₁₈-dihydrosphingosine (C₁₈-sphinganine), C₁₈-phytosphingosine (C₁₈-4*D*-hydroxysphinganine) and C₂₀-phytosphingosine, listed in order of increasing concentration. Weinert *et al.* (1973) investigated three ceramides from *S. cerevisiae*. In addition to the bases mentioned above, they discovered a large amount of mono-hydroxy and dihydroxy fatty acids as well as a relatively high content of C₁₉-phytosphingosine. Inositol-containing phosphosphingolipids of *S. cerevisiae* have been further studied by Steiner *et al.* (1969) and Steiner & Lester (1972). The major sphingolipid had the composition ceramide-(*P*-inositol)₂-mannose, and at least four other inositol-containing phosphosphingolipids were detected.

Experimental

Materials

The commercial baker's yeast (*S. cerevisiae*) was obtained from the Rajamäki Factories of the Finnish State Alcohol Monopoly, Rajamäki, Finland. The preparation methods of whole cell envelopes and clean cell walls have been described (Suomalainen *et al.*, 1967; Nurminen *et al.*, 1970).

The ion-exchange resins used were Chelex 100 (100–120 mesh) (Bio-Rad Laboratories, Richmond, Calif., U.S.A.), Dowex 50W (X8; 200–400 mesh) (J. T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.) and Amberlite IR-4B (OH⁻ form) (BDH Chemicals Ltd., Poole, Dorset, U.K.). Silicic acid (Unicil; Clarkson Chemical Co. Inc., Williamsport, Pa., U.S.A.) was used for column chromatography of the lipids. The t.l.c. absorbents were silica gel G and HF₂₅₄ (E. Merck A.G., Darmstadt, Germany). Ready-made plates were coated with silicagel 60 F₂₅₄ (DC-Fertigplatten; E. Merck A.G., Darmstadt, Germany) for preparative t.l.c. Except for phytosphingosine (Makor Chemicals Ltd., Jerusalem, Israel), lipid standards were purchased from Applied Science Laboratories Inc., Pa., U.S.A.

Isolation of total lipids

Lipids were isolated by chloroform-methanol extraction by the method of Trevelyan (1966) and washed with aq. NaCl solution (Folch *et al.*, 1957).

Column and thin-layer chromatography

After the Folch partition, acidic lipids were changed to sodium salts with Chelex 100 ion-exchange resin (Renkonen, 1968). Lipids were eluted from the resin with chloroform-methanol-water (5:4:1, by vol.)

and the solvents evaporated. The lipid mixture in chloroform was applied to a silicic acid column (1g of Unisil/10mg of lipid was used) and eluted with chloroform, chloroform–acetone, acetone and acetone–methanol mixtures (Rouser *et al.*, 1967; Vance & Sweeley, 1967; Kwiterowich *et al.*, 1970).

The neutral lipids were eluted first with chloroform (solvent C, 1500ml), and neutral glycolipids with chloroform–acetone (solvent CA, 1:1, v/v, 1000ml) and acetone (solvent A, 4000ml). Acetone–methanol mixtures (solvents: AM, 19:1, v/v, 1000ml; AM, 9:1, v/v, 2000ml; AM, 4:1, v/v, 1000ml; AM, 2:1, v/v, 1000ml) and methanol (solvent M, 2000ml) were used to obtain acidic glycolipids and the phosphoglycerides. The eluted fractions were weighed on a micro-balance and then examined by t.l.c. The solvent systems were chloroform–methanol (9:1, v/v) for glycolipids and chloroform–methanol–water (65:25:4, by vol.) for samples containing phospholipids. The detection reagents used on the thin-layer chromatograms were aniline–diphenylamine–phosphoric acid for reducing sugars (Bailey & Bourne, 1960), molybdenum–H₂SO₄ reagent (Dittmer & Lester, 1964) for phospholipids and aq. 20% ammonium bisulphate as a common charring reagent for all lipids (Privett *et al.*, 1961).

Analytical methods

Two different acid-hydrolysis methods were used to elucidate the sugar composition of yeast glycolipids: aq. 2M-HCl (3h, 100°C; Pepper & Jamieson, 1969; Lopes & Innis, 1970) or Dowex 50W (X 8; H⁺ form) in 0.01M-HCl (about 30h, 100°C; Lehnhardt & Winzler, 1968). The only detectable difference between the methods was a slightly higher recovery of galactose and mannose from the second procedure. After hydrolysis, fatty acids were extracted with chloroform, and the aqueous phase was neutralized in basic anion-exchange resin (Amberlite IR-4B, regenerated with 5% Na₂CO₃ in water; Sjöström, 1971). Sphingosine bases were extracted after the addition of sodium metaborate (to pH 10–11; Sjöström *et al.*, 1966). Sugars in the aqueous phase were reduced with NaBH₄. The solution was then deionized in cation-exchange resin (Dowex 50W; X 8; H⁺ form) and borate was removed by evaporating three times to dryness from methanol. Sugars were acetylated with acetic anhydride–pyridine (1:1, v/v; 6h at 100°C) for g.l.c. Standard sugars were reduced and derivatized in the same way.

Neutral sugars in the lipid fractions were examined as their alditol acetates by g.l.c. by using 3% ECNSS-M on Gas-Chrom Q (100–120 mesh) (Björndahl *et al.*, 1967; Borchardt & Piper, 1970; Kim *et al.*, 1967). The column temperature was programmed from 155° to 170°C at 2°C/min, held isothermally for 1min and then programmed from

170° to 195°C at 1°C/min. The instrument used for g.l.c. was a Hewlett–Packard 7620A Research Chromatograph equipped with a flame ionization detector. He was used as carrier gas for all analyses. The percentage composition of the neutral sugars was calculated from the relative peak areas. Quantitative measurement of reducing sugars was performed with phenol–H₂SO₄ by the method of Dubois *et al.* (1956).

The column used for the g.l.c. of fatty acids was 2% SE-52 on Gas-Chrom Q (100–120 mesh). Because the procedure and results were essentially the same as in our earlier work (Nurminen & Suomalainen, 1971), they are not reported here.

For the examination of sphingosine bases, lipids were hydrolysed in aq. methanolic 1M-HCl (18h, 70°C) by the method of Gaver & Sweeley (1965). Bases were extracted from the hydrolysate with chloroform and trimethylsilylated for g.l.c. (Carter & Gaver, 1967). The column used for the analyses was 3.8% SE-30 on Supelcoport [80–100 mesh; 185cm×0.635cm (6ft×¼in)]. Sphingosine base derivatives were identified by co-chromatography with standard bases (isothermally at 210°C) or from plots of the logarithm of retention time against carbon number. Percentage compositions were calculated from the relative peak areas. Quantitative analyses were performed by the spectrophotometric method of Lauter & Trams (1962).

After the acidic hydrolysis of sterol glycosides, the sterols and fatty acids were extracted in chloroform, and the fatty acids were separated from sterols by saponification with 0.5M-KOH in methanol (Johnston *et al.*, 1957). The sterols were analysed as their acetates by g.l.c. on a column of 1% SE-30 on Gas-Chrom Q (100–120 mesh) used isothermally at 210°C. The percentage composition of the different sterols was calculated from the corresponding peak areas. Sterols were measured quantitatively by a modified Lieberman–Burchard spectrophotometric method (Sackett, 1925) with ergosterol as a standard.

Sulpholipids were determined with Kean's specific and sensitive spectrophotometric method (Kean, 1968) with ox brain sulphatides as a standard.

Results

Column and thin-layer chromatography of lipids

The lipid fractions from the silicic acid column were weighed on a micro-balance to obtain a primary quantification. Table 1 shows the weights of the lipids eluted. Glycolipids recovered from silicic acid were identified on thin-layer chromatograms by means of reference compounds and specific detection reagents (Plates 1, 2 and 3). Some glycolipids were detected among the neutral lipids with the aniline–diphenylamine–phosphoric acid reagent for reducing sugars.

EXPLANATION OF PLATE I

Neutral cell-envelope glycolipids of baker's yeast eluted from silicic acid

The solvent system for t.l.c. was chloroform-methanol (9:1, v/v). Lipids were detected by charring with aq. 20% ammonium bisulphate. Reference compounds were monogalactosyl diglyceride (MG), ox brain cerebroside (Cer) and sulphatide (Su). The thin-layer plate was precoated with silica gel HF₂₅₄. For details of solvent systems [CA, C(3) and A] and the numbered spots see the text.

EXPLANATION OF PLATE 2

Phospholipids and acidic glycolipids in cell envelopes of baker's yeast recovered from silicic acid

The solvent system used for t.l.c. was chloroform-methanol-water (65:25:4, by vol.). Lipids were detected with aq. 20% ammonium bisulphate. Reference compounds were ox brain cerebroside (Cer) and sulphatide (Su). A commercial precoated silica gel HF₂₅₄ plate was used. PI, Phosphatidylinositol. For details of the solvent systems (AM and M) and the numbered spots see the text.

EXPLANATION OF PLATE 3

Phospholipids and acidic glycolipids in cell envelopes of baker's yeast eluted from silicic acid

The solvent system used for t.l.c. was chloroform-methanol-water (65:25:4, by vol.). Glycolipids were detected with aniline-diphenylamine-phosphoric acid (85%) reagent for reducing sugars. The thin-layer adsorbent was silica gel G. Reference compounds were as for Plate 1. For details of the solvent systems (AM and M) and the numbered spots see the text.

Table 1. *Distribution of total lipids from the cell envelopes of baker's yeast in different elution mixtures from a silicic acid column (70g)*

Recovery was 98.2% and the amount of total lipid 696mg.

Fraction	Vol. (ml)	Amount of lipids:	
		(mg)	(% of total)
Neutral lipids:			
Chloroform C(1)	300	395.9	56.9
C(2)	200		
C(3)	1000		
Glycolipids:			
Chloroform-acetone (1:1, v/v)	1000	27.5	4.0
Acetone	4000	9.1	1.3
Phospholipids and the remaining glycolipids:			
Acetone-methanol (19:1, v/v)	1500	3.0	0.4
Acetone-methanol (9:1, v/v)	2000	11.4	1.6
Acetone-methanol (4:1, v/v)	1000	39.3	5.6
Acetone-methanol (2:1, v/v)	1000	53.6	7.7
Methanol	2000	144.0	20.7

 Table 2. *Relative amounts of envelope sterols of baker's yeast (calculated from peak areas) in chloroform and chloroform-acetone fractions after the main part of neutral lipids was eluted with 300 ml of chloroform*

Retention times were calculated in relation to cholesterol (1.0). For details of solvent fractions see the text.

Component	Relative retention time	Fraction		
		C(2) (200ml) (%)	C(3) (1000ml) (%)	CA (1:1, v/v; 1000ml) (%)
Cholesterol	1.0	5.6	5.1	3.0
Zymosterol	1.13	20.9	18.0	5.5
Ergosterol	1.22	44.0	24.4	33.7
Episterol	1.43	13.8	17.9	2.7
Lanosterol	1.58	6.7	7.1	0.8
Unknown compound 1	1.77	7.3	14.1	35.0
Unknown compound 2	2.03	1.7	13.4	19.0
Lieberman-Burchard determination (μ g of sterol/g dry wt. of cell envelope)		37.5	8.8	184

Fractions eluted with chloroform-acetone and acetone revealed no phospholipids with molybdenum- H_2SO_4 reagent. Not all the neutral glycolipids, however, were eluted with these solvents: the remainder were detected in the first methanol-containing fractions together with the phospholipids. Cerebrosides and sulpholipids could be recognized on the thin-layer chromatograms by comparison with reference compounds. The characteristic red and blue colours of sterol glycosides appeared when the chloroform-acetone fraction was sprayed with aq. 50% H_2SO_4 (Heftmann *et al.*, 1966). The main component (spot 8, Plate 1) appears on the thin-layer chromatogram just after the monogalactosyl diglyceride standard. Several other sterol glycosides were also detected in small amounts by the Lieberman-Burchard spectrophotometric method, including

sterol glycosides (spot 10, Plate 1, just above the upper spot of ox brain cerebroside) and possibly some acylsterol glycosides (between spots 3 and 6, Plate 1).

The largest amount of yeast glycolipids was found to be eluted among and after the phosphatidyl diglycerides. On the thin-layer chromatograms (Plates 2 and 3) the main part of the sulpholipids was detected together with phosphatidylinositol and a small amount (spot 3) above it. There are several components among the sphingolipids containing phosphate, some of which remain at the starting point with the solvent mixture used.

Neutral glycolipids

Several lipids could be detected by t.l.c. (Plate 1) in the third chloroform [solvent C(3)] and chloroform-acetone (solvent CA 1:1, v/v) eluates from the

silicic acid column, but efforts to isolate individual lipids in a pure form resulted in a lot of difficulties. Consequently, the fractions from the silicic acid column were each treated as a whole to elucidate the composition of sterols, sphingosines and sugars by g.l.c. For an additional identification of glycolipids in these eluates from silicic acid, some samples were subjected to mild alkaline deacylation (Renkonen, 1963) and then run on a thin-layer plate to detect any glycosidic cleavage of glycolipids. The Lauter-Trams method (Lauter & Trams, 1962), Lieberman-Burchard determination (Sackett, 1925) and phenol-H₂SO₄ were used to identify respectively sphingosines, sterols and reducing sugars. Samples were run with chloroform-methanol (9:1, v/v); the zones were detected with water and scraped off. Lipids were eluted from silica gel with chloroform-methanol (2:1, v/v) by using the procedures described above.

The following conclusions can be drawn from these results. The dominating lipids in the eluates are ceramides and sterol glycosides. In Plate 1, spots 1, 3, 5, 7 and possibly 9 are ceramides containing a lot of sphingosine and no reducing sugars. Spots 8 and 10 at least are sterol glycosides. On the basis of the sugar determinations, alkaline stability and the absence of sphingosine, spots 2 and 6 are probably acyl sugars. A relatively large amount of some alkali-labile compound (spot 4) that continued to be eluted among the phospholipids was in the acetone fraction (solvent A).

The composition of sterols derived from acidic hydrolysis of sterolglycosides was investigated by g.l.c. (Patterson, 1971). The chloroform fractions C(2) and C(3) were examined to compare the sterols in the last neutral lipids eluted from silicic acid with the sterols among the glycolipids (Table 2). The main part of the neutral lipids had previously been eluted with the same solvent. The percentage of unidentified sterols was higher in the sterol glycosides.

Long-chain bases in the neutral glycolipids are presented in Table 3. A high amount of sphingosines (24–27%) indicates the presence of ceramides, as does the quite low amount of sugars (Table 4). G.l.c. of the neutral sugars (Table 5) revealed the presence of glucose, mannose, galactose and traces of rhamnose. Acylglucoses, sterol glycosides and sulpholipids may contain glucose, and cerebroside probably contain galactose. A small quantity of sulpholipids was detected by Kean's (1968) specific method (Table 6).

Acidic glycosphingolipids

Sulpholipids were eluted principally with eluents containing methanol (Table 6). They had not been isolated from phospholipids, but besides glucose they could contain galactose (Table 5), which has not previously been detected in phosphosphingolipids.

Table 3. Quantitative amounts (Lauter-Trams method) and percentages of different long-chain bases in the cell-envelope lipids baker's yeast eluted from a silicic acid column

For details of solvent fractions C, CA, AM, A and M see the text.								
Long-chain base	Relative retention time at 210°C	Fraction	Amount of long-chain bases (% of total area)					Total amount
			... C(2) and C(3)	CA (1:1, v/v)	A	AM (19:1, v/v) and (9:1, v/v)	AM (4:1, v/v) and (2:1, v/v) and M	
With chain length C ₁₄ –C ₁₇	0.25–0.65			1.82	0.66	0.44	2.42	5.87
C ₁₈ -sphingosine	0.85			0.20	0.08	0.05		0.40
C ₁₈ -dihydrosphingosine	1.00			1.52	2.42	0.85	2.97	6.80
	1.14			0.08	0.06	0.04		0.18
C ₁₉ -dihydrosphingosine	1.31		0.03	0.10	0.06	0.05	1.77	2.00
C ₁₈ -phytosphingosine	1.48			17.46			38.12	55.58
C ₂₀ -sphingosine	1.81						0.49	0.49
C ₂₀ -dihydrosphingosine	2.01						0.37	0.37
C ₂₀ -dehydrophytosphingosine	2.47						6.89	9.05
C ₂₀ -phytosphingosine	2.79		0.47	0.79	0.37	0.53	16.77	19.26
Totals		...	1.82	24.46	1.99	1.96	69.79	100.00
			0.2	27.3	1.2	1.0	70.3	100.0
			2.8	431.6	18.4	15.2	1109.4	1577.4
Lauter–Trans method: (%)								
(nmol/g dry wt. of cell envelopes)								

Lauter-Trams method: (%)
(nmol/g dry wt. of cell envelopes)

Table 4. *Amounts of reducing sugars in cell-envelope lipids of baker's yeast eluted from silicic acid*

Reducing sugars were measured by the method of Dubois *et al.* (1956). Results were calculated with glucose as standard.

Fraction	Amount of reducing sugars:	
	(μg of glucose/g dry wt. of cell envelope)	(%)
Chloroform C(2)	0.54	1.7
Chloroform C(3)	0.50	1.5
Chloroform-acetone (1:1, v/v)	1.03	3.2
Acetone	1.62	5.0
Acetone-methanol (19:1, 9:1, 4:1 and 2:1, all v/v) and methanol	18.82	88.6
Totals ...	32.50	100.0

Table 5. *Content of rhamnose, mannose, galactose, glucose and inositol in the cell-envelope lipids of baker's yeast eluted from silicic acid*

Experimental details are given in the text. The results are expressed as percentage of the total amount obtained from 1 g dry wt. of cell envelopes. tr., Below 0.1%. For details of solvent fractions see the text.

Component	Fraction					Sum of fractions
	C(2) and C(3)	CA (1:1, v/v)	A	AM (19:1, v/v) and (9:1, v/v)	AM (4:1, v/v) and (2:1, v/v) and M	
Rhamnose	tr.		tr.	tr.		tr.
Mannose		0.1	0.1	0.1	14.7	15.0
Galactose	tr.	0.1	tr.	tr.	0.2	0.3
Glucose		0.9	0.4	0.6	1.2	3.1
Inositol				0.2	81.4	81.6
Totals	tr.	1.1	0.5	0.9	97.5	100.0

Table 6. *Quantitative amounts of sulpholipids in cell-envelope lipids of baker's yeast eluted from silicic acid*

Fraction	Amount of sulpholipid	
	(nmol of sulphatide/g dry wt. of cell envelope)	(%)
Chloroform-acetone (1:1, v/v)	15.8	2.8
Acetone	40.6	7.2
Acetone-methanol (19:1, v/v) and (9:1, v/v)	20.7	3.7
Acetone-methanol (4:1, v/v) and (2:1, v/v)	245.0	43.6
Methanol	240.0	42.7
Totals	562.1	100.0

Although phosphosphingolipids mainly contain inositol and mannose, the very high amount of inositol found (Table 5) is partly due to phosphatidyl-inositols, which have been shown to be concentrated in the plasma membrane isolated from the cell envelopes of baker's yeast (Suomalainen & Nurminen, 1970).

Phosphosphingolipids with a complex structure were further isolated from phosphatidyl diglycerides

by t.l.c. A mild alkaline hydrolysis was performed to detect lysophosphatides or di- and tri-phosphoinositides, and accordingly three alkali-labile compounds are seen (spots 3, 5 and 6 in Fig. 1). The several compounds still remaining support the findings of Steiner & Lester (1972), who identified the main compound, ceramide-(*P*-inositol)₂-mannose, and at least four other inositol-containing phosphosphingolipids in the lipids from the whole cells of *S. cerevisiae*.

Fig. 1. Cell-envelope phosphosphingolipids of baker's yeast isolated from phosphatidyl diglycerides

The solvent systems for two-dimensional t.l.c. were (1) chloroform-methanol-aq. 25% NH_3 -water (50:25:6:1.5, by vol.) and (2) chloroform-methanol-water (50:25:3.8, by vol.). Lipids were detected with aq. 20% ammonium bisulphate. The thin-layer plate was precoated with silica gel HF₂₅₄. 1-10 refer to the eluted compounds, for details see Plates 1-3 and the text.

Discussion

We investigated the cell-envelope fraction of baker's yeast to obtain information about the location and function of glycolipids in the plasma membrane and cell wall. Accordingly, we also investigated the lipids of a thoroughly washed cell-wall preparation that contains no plasma membrane (Suomalainen *et al.*, 1967; Nurminen *et al.*, 1970). Although the experiment was performed on only a small scale, we noticed at least a small amount of sterol glycosides and phosphosphingolipids containing inositol and mannose. Inositol-containing phospholipids have long been known to constitute the main part of yeast glycolipids. The occurrence of these inositol-containing glycolipids in organisms possessing cell walls is significant because of their possible role in the production and function of the cell wall. Compared with a preliminary investigation of lipids in whole cells, a somewhat higher concentration of complex phosphosphingolipids and alkali-labile compounds eluting with acetone were detected in cell envelopes.

It would be interesting to obtain more information about the unidentified compounds present in relatively high amounts among the sterols in the unsaponifiable fraction. If they are sterols, it is difficult to understand why they should be concentrated with the sterol glycosides.

The occurrence of phytosphingosines is ubiquitous in bacteria, fungi and plants. Stoffel (1973) suggests that the phytosphingosines that may occur in the sphingolipids of animal tissues are derived from exogenous (plant) sources. Moreover, it has been proposed that the biosynthesis of sphingosine and dihydrosphingosine is different in animals and yeast. Biosynthesis of dihydrosphingosine in yeast from palmitoyl-CoA and serine (pyridoxal-*P*-enzyme) proceeds via a 3-oxo intermediate, 3-oxodihydrosphingosine. The same kind of intermediate, identified as 3-oxosphingosine, has been found in the biosynthesis of sphingosine from *trans*-2-hexadecenoyl-CoA (Brady *et al.*, 1969). The third hydroxyl group of phytosphingosine could not be shown to originate from α -hydroxypalmitoyl-CoA; phytosphingosine is probably synthesized from dihydrosphingosine. The biosynthesis of saturated and unsaturated bases in yeast follows parallel pathways. Further, DiMari *et al.* (1971) proposed that the occurrence of sphingosine in only traces in yeast is due to restricted accessibility of fatty acyl-CoA derivatives other than palmitoyl-CoA.

Our results confirm the prevalence of C_{18} -phytosphingosine. In addition, critical micellar concentrations of acyl-CoA homologues affect the inhibition of the condensing enzyme. The inhibition effect increases with increasing chain length. Because of its low critical micellar concentration palmitoyl-CoA favours the synthesis of long-chain bases. However, the frequent predominance of C_{20} -phytosphingosine in previous investigations with whole yeast cells (Wagner & Zofcsik, 1966; Weinert *et al.*, 1973) may also be due to a more complete isolation method than ours; this base linked to an oligosaccharide and possibly a hydroxy fatty acid forms a quite polar compound. The occurrence of C_{20} -dehydrophytosphingosine in yeast has not been reported, but in support we have also detected a small amount of its precursor, C_{20} -sphingosine. The presence of C_{19} -dihydrosphingosine has not been reported either, but the succeeding compound in biosynthesis, C_{19} -phytosphingosine, has been demonstrated in yeast ceramides by Weinert *et al.* (1973).

The trace amounts of rhamnose present among neutral lipids and glycolipids suggest the possible occurrence of bacterial-type rhamnolipids in yeast. Like acylglucoses, they are composed of fatty acids glycosidically linked to carbohydrates. Brennan *et al.* (1970) detected acylglucoses in *S. cerevisiae*. The presence of glycolipids containing glycerol instead of long-chain bases was tentatively proposed by

Baraud *et al.* (1970), who identified by t.l.c. monogalactosyl diglycerides and sterol glycosides, besides sulphatides, in the lipids of *S. cerevisiae* after DEAE-cellulose column chromatography. In our investigations small amounts of similar compounds could be detected in the cell-envelope lipids of baker's yeast.

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