fered (pH 6) medium is preferred over the non-buffered one. The data also indicate that 30 °C is also preferred for incubation than 37 °C. If the assay is performed at optimal conditions (slightly acidic medium and at 30 °C incubation) diluted solutions of ceftizoxime retain their original potency at 4 °C for 3 months. In keeping with this premise, ceftizoxime was found to be a very stable cephalosporin in aqueous solution for a long period of time.

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Occurrence of 3-methoxy octadecanoic acid in yeast lipid[1]

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Summary. The fatty acid composition of a new strain of the yeast *Rhodotorula glutinis,* grown in molasses, has been studied and found to contain palmitic, stearic, oleic, linoleic and linolenic acids, and small amounts of other constituents. In addition, 3-methoxy octadecanoic acid has been shown to be present in the glycolipid fraction.

The fatty acid compositions of numerous fungal species have been described since the introduction of gas chromatography. Besides the typical fatty acids such as palmitic, stearic, oleic, linoleic and linolenic acids, unusual fatty acids with hydroxy, keto and epoxy functions have also been reported[5].

Several yeasts and yeast-like fungi produce extracellular hydroxy or acetylated long chain fatty acids. For example, 3-D-hydroxy-palmitic acid has been identified as a product of *Saccharomyces malanga*[6]. Acetylated fatty acids such as 8,9,13-triacetoxydocosanoic and 13-oxo-8,9-diacetoxydocosanoic acids have been isolated as products of a yeast-like fungus closely related to *Torulopsis fujisanesis.* Partially acetylated and esterified 8,9,13-trihydroxydocosanoic acid has been isolated from a *Rhodotorula* species[7]. The presence of 17-L-hydroxyoctadecanoic and 17-L-hydroxyoctadecenoic acids have been confirmed in the extracellular glycolipids from *Torulopsis magnaliae*[8] and 13-hydroxydocosanoic was found in the extracellular glycolipid of *Candida bogoriensis*[9]. Recently, 7-methoxytetradec-4(E)-enoic acid[10] and 7-methoxy-9-methylhexadeca-4(E), 8(E)-dienoic acid[11] have been isolated from the marine blue gree alga *Lyngbya majuscula.*

A preliminary study in a search for a yeast producing high levels of oil led to a new isolate of *Rhodotorula glutinis* from soil[12]. Besides the typical fatty acids, this oleaginous yeast strain contained a novel methoxy fatty acid in the glycolipid fraction. The present report describes the production, isolation and characterization of this hitherto unreported acid.

Materials and methods. The primary criterion for selecting the yeast isolate used in this study was its ability to survive and metabolize under nitrogen-deficient conditions, producing fat in high yield[13]. Characterization of the genus and species of this red yeast isolate was carried out according to the procedure of Van der Walt[14]. Finally, the strain was

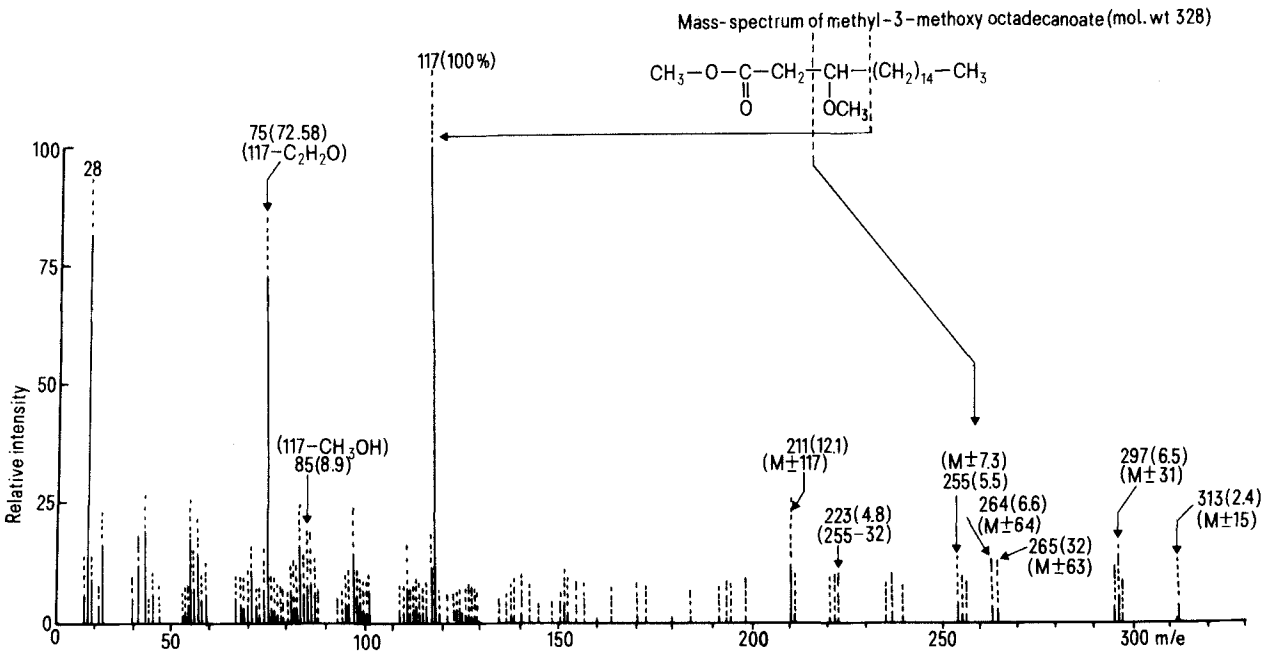

Figure 1. Gas chromatograms of the methyl esters of the fatty acids of the glycolipid fraction of *R. glutinis* lipid, on 10% DEGS column. Oven temperature, 180 °C; detector and injection port temperatures, 250 °C. Nitrogen flow rate, 60 ml/min.

allowed to grow for 42 h in a medium containing 42% (w/w) of sugar, 1.8% (w/w) of nitrogen and 0.86% (w/w) phosphorus.

The lipid was extracted from dry cells according to the method of Bligh and Dyer[15] as modified by Kates[16]. The dry cells were homoginized with methanol-chloroform-water (2:1:0.8, v/v), centrifuged and the solvent layers separated. The residue was then extracted twice in the same way. The extracts were pooled and washed thrice with water and dried over anhydrous sodium sulphate. The solvent was evaporated in a rotary evaporator under vacuum at 40 °C. A portion of the total lipid was saponified[17] by refluxing with methanolic KOH. The nonsaponifiable substances were removed by washing thrice with diethyl ether. The fatty acids were removed from the hydrolysate after acidifying with 4N H_2SO_4, and dried over anhydrous Na_2SO_4. The ether was evaporated in a rotary evaporator under vacuum at 30 °C. The fatty acids were methylated with diazomethane[18].

A portion of the total lipid was fractionated by column chromatography on silicic acid[19]. The neutral, glyco-and phospholipids were eluted with chloroform, acetone and methanol respectively. The fractions thus obtained were saponified and the methyl esters of the fatty acids were prepared as described previously. The methyl esters were first analyzed by TLC using a solvent system of light petroleum ether (40–60 °C) and diethyl ether (80:20, v/v). The spots were visualized under UV-light after spraying the plate with 1% (w/v) of 2',7'-dichlorofluorescein in ethanol. Fatty acid methyl esters of the glycolipid fraction were subjected to column chromatography on silicic acid. The column was washed with a solvent system consisting of light petroleum ether (40–60 °C) with increasing proportions of diethyl ether. The content of fractions from the silicic acid column was monitored by analytical gas chromatography. The fraction eluted by 50% diethyl ether contained a major component with an equivalent chain length (ECL) value[20] of 21.6 determined by analytical gas chromatography. This fraction was further purified by preparative gas chromatography.

Analytical and preparative gas chromatography were performed using Pye Unicam instruments, models GCD and 104 respectively, equipped with flame ionisation detectors. Glass columns were packed with diethylene glycol succinate polyester (DEGS) as liquid phase, supported on Chromosorb-W (HP). Typical fatty acids were identified by comparing their retention times with those of authentic standards. Identification of the unsaturated acids was confirmed by catalytic hydrogenation[22] of portions of methyl ester samples. Quantitation from chromatograms was done by triangulation of the peaks and the internal normalisation technique[23]. PMR-spectra of the compounds were recorded in $CDCl_3$ on a Varian 90 MHz spectrometer, model EM-390. The mass-spectra were recorded on a low resolution AEI mass spectrometer model MS-30, with an ion voltage of 70 ev.

Results and discussion. TLC of the fatty acid methyl esters of the glycolipid fraction gave 2 spots, one corresponding to the methyl esters of typical fatty acids with R_f, 0.65 and the other at R_f, 0.40. Analytical gas chromatography of the methyl esters of the glycolipid fraction showed, besides typical fatty acid methyl esters, as presented in figure 1, a peak with an equivalent chain length value of 21.6. Column chromatography of the fatty acid methyl esters of the glycolipid fraction yielded a fraction with 50% diethyl ether which contained the above mentioned compound as the major component (85%) as revealed by GLC. This major compound was purified by preparative gas chromatography and was found to be pure by analytical gas chromatography criteria.

The PMR-spectrum of the compound isolated (fig. 2) showed singlets at δ 3.7, 3.3 and 1.2 due to the protons of -$COOCH_3$, -OCH_3 and -CH_2-respectively[24]. The terminal methyl protons appeared as a triplet[24] centered at δ 0.85. The integration curve showed the presence of 3 protons each for -$COOCH_3$, -OCH_3 and -CH_3 groups. A partially resolved signal at δ 2.45 was due to the 2 protons at α-carbon to the carboxyl group.

The MS of the compound is presented in figure 3. The base peak is at 117. There was no molecular ion peak in the MS. As revealed from the PMR-spectrum, the fatty acid in question contains an -OCH_3 group; the molecular ion peak

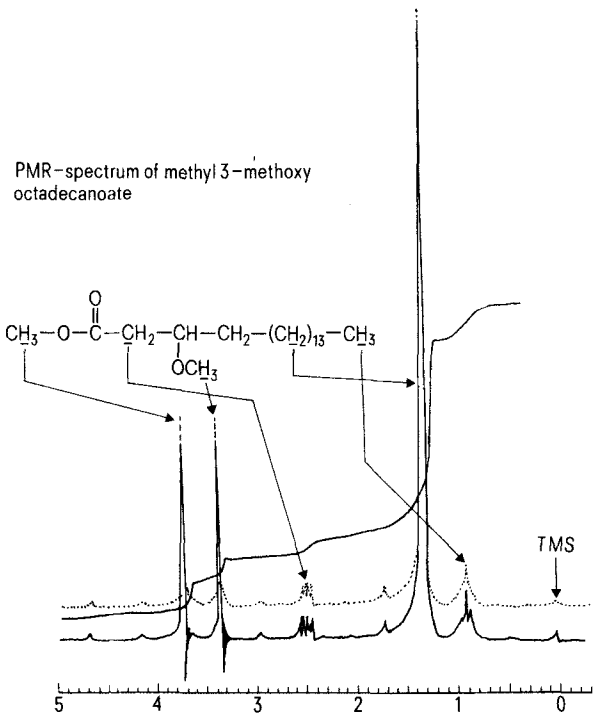

Figure 2. PMR-spectrum of methyl-3-methoxyoctadecanoate. From *R. glutinis* lipid.

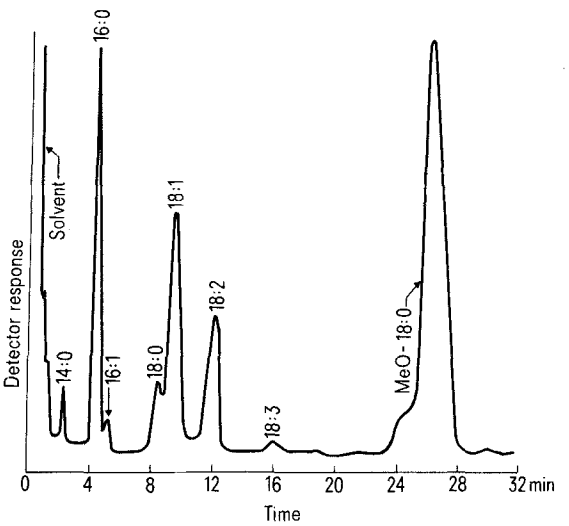

Figure 3. Mass-spectrum of methyl-3-methoxyoctadecanoate. From *R. glutinis* lipid.

Fatty acid compositions[d] of various lipids fractions of *Rhodotorula glutinis*

Component acids[a]	Neutral[b] lipid (97%)	Glycolipid[b] (2%)	Phospholipid[b] (1%)	Total lipid[c] (55%)
12:0	1.0	–	–	1.2
14:0	2.1	0.9	0.9	1.5
16:0	32.7	10.8	30.0	35.0
16:1	5.5	0.8	2.9	0.8
18:0	8.2	2.9	1.6	2.5
18:1	32.2	10.5	27.7	44.0
18:2	16.6	6.5	29.0	7.0
18:3	1.7	0.6	7.9	2.0
MeO – 18:0	–	67.0	–	6.0

[a] 1st and the 2nd figures represent, carbon number:number of double bond. [b] Expressed as percent w/w of total lipid. [c] Expressed as percent w/w of dry cell. [d] Expressed in mole percents.

should be absent or very low in esters of this type[25]. Loss of methanol[25] from fragment 117 leads to the fragment 85 (8.9%). The fragment 75 (72.6%) was produced by the loss of ketene[26] (C_2H_2O) from the fragment 117. The presence of 2 methoxyl groups characteristically leads to a doublet[25] of small abundance due to $M^+ - 63$ ($OCH_3 + CH_3OH$) and $M^+ - 64$ ($2 \times CH_3OH$) fragments; this allowed us to calculate[25] the mol. wt, which is 328. Presence of fragment 313 (3%) was due to $M^+ - CH_3$ which is common in fatty acid methyl esters. Other important fragments were 255 (6%) due to 2,3-cleavage and 233 due to the loss of methanol[25–27] from fragment 255.

The PMR- and MS-data suggest that the compound should be methyl-3-methoxyoctadecanoate. Finally, superimposable PMR (fig. 2) and MS (fig. 3) were obtained with those of authentic methyl-3-methoxyoctadecanoate, prepared by methylating[28] methyl-3-hydroxy-octadecanoate, obtained from Applied Science Laboratories Inc., Pa, USA.

The fatty acid composition of neutral, phospho-, glyco- and total lipids of *R. glutinis* is presented in the table. The fatty acid composition of total lipid shows that it is rich in palmitic (16:0) and oleic (18:1) acids, which is also true of the neutral lipid fraction. The phospholipid fraction is rich in palmitic, oleic and linoleic (18:2) acids. The glycolipid fraction is very unusual regarding its fatty acid composition, bacause of the presence of a high proportion (67%) of the methoxy acid. It is interesting to note that the occurrence of this methoxy fatty acid is restricted to the glycolipid fraction.

In earlier studies on the fatty acid composition of *R. glutinis* by Kates et al.[27], major constituents were oleic (58%) and stearic (14%) acids. Kaneko et al.[30] found linoleic (53.1%)oleic (31.6%) and palmitic (12.3%) acids as major constituents of *R. glutinis* lipid.

The lipid content and fatty acid composition are largely dependent on various factors like temperature, carbon source, nitrogen, phosphorus and vitamins[5], and it is very likely that different growth conditions would cause variations in the fatty acid make-up of the same organism. In the earlier studies[29,30] common culture media, like YM-culture medium was used which contained enough carbon and nitrogen, whereas in the present study just sufficient carbon and nitrogen was used for the production of fat in high yield[13].

1 Partly presented at the National Symposium on Natural Product Chemistry held on February 7–8, 1983 at Bose Institue, Calcutta (India).
2 Acknowledgments. Authors are indebted to Prof. S.C. Bhattacharyya, and Prof. A.K. Barua, Department of Chemistry for providing facilities for this work.
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