2. A. D. Turova and A. S. Gladkikh, Farmakol. Toksikol., 28, 428 (1965).
3. L. T. Bogomolov and V. K. Sharkov, Probl. Gematol. Pereliv. Krovi, 2, 59 (1958).
4. R. S. Schweiger, J. Agr. Chem., 29, 2973 (1966).
5. A. D. Bezzubov, A. I. Khatina, and O. E. Vasil'eva, Gig. Truda Profzabol., 3, 125 (1960).
6. A. D. Bezzubov and A. I. Khatina, Gig. Truda Profzabol., 7, 16 (1961).
7. S. Lipinskii, Gig. Truda Profzabol., 4, 68 (1961)..
8. A. D. Bezzubov, Izobretatel'stvo v SSSR, 5, 89 (1956).
9. R. Kohn and V. Tibensky, Coll. Czech. Chem. Commun., 36, 1 (1971).
10. G. B. Aimukhamedova, Z. D. Ashubaeva, and É. A. Umaraliev, The Chemical Modification of Pectin Substances, Frunze (1974), p. 37.
11. V. M. Ropot, I. I. Skripnik, and M. P. Filippov, Izv. Akad. Nauk MSSR, Ser. Biol. Khim. Nauk, No. 1, p. 72 (1972).
12. C. Tanford, Physical Chemistry of Macromolecules, Wiley, New York (1961) [Russian translation, Moscow (1965), p. 772].
13. S. P. Afanas'ev, É. P. Panova, G. N. Katseva, E. P. Kukhta, V. Ya. Chirva, Khim. Prir. Soedin., 428 (1984).
14. O. A. Songina and V. A. Zakharov, Amperometric Titration [in Russian], Moscow (1979), p. 304.
15. M. P. Filippov, Infrared Spectra of Pectin Substances [in Russian], Kishinev (1978), p. 76.

CHANGE IN CHEMICAL COMPOSITION OF TRIACRYLGLYCEROLS OF *Rhodotorula gracilis* AS A FUNCTION OF SPECIFIC GROWTH RATE

L. V. Zaitseva, Yu. A. Sultanovich, and A. P. Nechaev

UDC 547.915:663.14

The position-type composition of yeast triacylglycerols is represented mainly by types that are also characteristic for plant oils. To obtain unsaturated triacylglycerols it is better to use the continuous cultivation of yeast and not batch cultivation.

There are adequate grounds for considering that lipids of microbial origin and, in particular, yeast lipids are potential substitutes for plant oils used for technical demands. However, in addition to having a high productivity, lipid-forming strains of yeast produce lipids close in their chemical composition to solid plant fats. The main fraction in the lipid strains – active lipid-producing agents – just as in plant oils, consists of triacylglycerols (TAGs), the chemical composition and structure of which determine many of the physicochemical and technological characteristics of the lipids. We have previously studied the chemical compositions of the TAGs of several active lipid-forming strains belonging to various genera [14]. The biomass of these yeasts were obtained under the conditions of batch cultivation.

To obtain microbial lipids it is possible to use successfully the continuous method of cultivation, which ensures the more effective utilization of the organic substrates [2].

Our aim was to establish the stereotypic and molecular-species composition of the TAGs of yeast of various specific rates of growth (μ) of the culture.

We investigated the yeast strain *Rhodotorula gracilis* VKM u-335, which is capable of accumulating more than 40% of lipids on the dry mass of the cells under conditions of batch cultivation [3]. The investigations were performed in the regime of a chemostat with limitation of the growth of the culture by means of the source of nitrogen at rates of feed of medium to the fermenter of 0.1, 0.075, 0.05, and 0.1 h^{-1}, which under the conditions selected, corresponded to the specific rates of growth of the culture.

Moscow Technological Institute of the Food Industry. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 175-179, March-April, 1988. Original article submittted October 5, 1987.

 0009-3130/88/2402-0146$12.50

TABLE 1. Distribution of the Acyl Radicals in the TAGs of Rhodotorula gracilis as a Function of the Specific Rate of Growth (μ), Mole % on the Fatty Acids

μ, h^{-1}	Position	Fatty acid				
		16 : 0	18 : 0	18 : 1	18 : 2	18 : 3
0,1	TAGs	14,6	18,6	41,9	13,9	11,0
	sn-1	22,3	13,7	52,1	8,2	3,7
	sn-2	0,6	0,4	63,5	22,7	12,8
	sn-3	20,9	41,7	10,1	10,8	16,5
0,074	TAGs	15,6	20,3	46,0	10,1	8,0
	sn-1	26,6	18,2	41,9	7,8	5,5
	sn-2	0,6	0,3	71,8	17,7	9,6
	sn-3	19,6	42,4	24,3	4,8	8,9
0,05	TAGs	12,3	12 2	55,2	14,2	6,1
	sn-1	25,6	14,9	46,0	11,3	2,2
	sn-2	0,5	0,2	77,5	15,2	6,6
	sn-3	10,7	21,5	42,1	16,1	9,5
0,01	TAGs	24,9	8,2	50,8	11,1	5,0
	sn-1	34,5	15,9	39,2	7,6	2,8
	sn-2	1,0	0,5	76,5	15,6	6,4
	sn-3	39,2	8,2	36,7	10,1	5,8

TABLE 2. Stereotypic Composition and Main* Molecular Species of the TAGs of R. gracilis at μ = 0.1 h^{-1}

TAG†	Mole, %	TAG	Mole, %	TAG	Mole, %	TAG	Mole, %
SUS	21,86	SUU	13,84	UUS	39,57	UUU	23,59
Including:		Including:		Including:		Including:	
POP	2,95	POO	1,43	OOP	6,91	OOO	3,34
POSt	5,90	POL	1,53	LOP	1,09	OOL	3,57
StOSt	3,62	POLe	2,34	OOS	13,80	OLO	1,19
StOP	1,81	StOLe	1,44	OLSt	4,93	OOLe	5,46
PLSt	2,10	Others	7,10	OLP	2,47	OLL	1,28
StLSt	1,30			OLeSt	2,78	OLe	1,95
PLP	1,05			OLEP	1,39	OLeLe	1,10
PLeSt	1,18			LOSt	2,17	Others	5,70
Others	1,95			Others	4,03		

*Amounts of more than 1% on the total amounts of TAGs.
†Arbitrary symbols: S – saturated acyl; U – unsaturated acyl; P – palmitoyl; St – stearoyl; O – oleoyl; L – linoleoyl; Le – linolenoyl.

TABLE 3. Stereotypic Composition and Main* Molecular Species of the TAGs of R. gracilis at μ = 0.075 h^{-1}

TAG†	Mole, %	TAG	Mole, %	TAG	Mole, %	TAG	Mole, %
SUS	27,48	SUU	16,85	UUS	33,86	UUU	21,02
Including:		Including:					
POP	3,72	POO	4,64	OOP	5,90	OOO	7,31
POSt	8,10	StOO	3,18	OOSt	12,80	LOO	1,36
StOSt	5 54	PLO	1,14	OLP	1,45	OOL	1,44
StOP	2,56	POLe	1,70	LOP	1,10	OLO	1,80
PLST	2,00	StOLe	1,16	LeOSt	1,66	OOLe	2,68
StLSt	1,37	Others	5,03	LOSt	2,37	Others	0,43
PLeSt	1,08			OLSt	3,14		
Others	3,11			Others	1,71		
					3,73		

*Amounts of more than 1% of the total amount of TAGs.
†Arbitrary symbols the same as in Table 2.

TABLE 4. Stereotypic Composition and Main* Molecular Species of the TAGs of R. gracilis at $\mu = 0.05\ h^{-1}$

TAG†	Mole, %	TAG	Mole, %	TAG	Mole, %	TAG	Mole, %
SUS	13,52	SUU	27,11	UUS	18.89	UUU	39,88
including:		Including:		Including:		Including:	
		POO	8,35	OOP	3,81	OOO	15.00
POP	2,12	SOO	4,86	OOS	7,66	OOL	5,74
POS	4,27	POL	3.19	OLS	1,5	LOO	3,69
SOP	1 24	SOL	1,6	LOS	1,88	OLO	2,94
SOS	2,48	PLO	1,64	Others	4,04	LOL	1,41
Others	3,41	POLe	1 88			OOLe	1,13
		SOLe	1,10			OLeO	3,39
		Others	4 23			Others	1,28
							5,30

*Amounts of more than 1% of the total amount of TAGs.
†Arbitrary symbols the same as in Table 2.

TABLE 5. Stereotypic Composition and Main* Molecular Species of the TAGs of R. gracilis at $\mu = 0.01\ h^{-1}$

TAG†	Mole, %	TAG	Mole, %	TAG	Mole, %	TAG	Mole, %
SUS	23,51	SUU	26,07	UUS	23,26	UUU	25,74
Including:		Including:		Including:		Including:	
				OOP	11,80	OOO	11,00
POP	10,30	POO	9,69	OOS	2,46	LOO	2,13
POS	2,16	SOO	4 46	LOP	2,28	OOL	3.03
SOP	4,77	POL	2,67	OLP	2,40	OLO	2,24
SOS	1,00	PLO	1,98	Others	4,32	OOLe	1,74
PLP	2,11	SOL	1,23			Others	5,60
SLP	1,00	POLe	1,53				
Others	2,17	Others	4,51				

*Amounts of more than 1% of the total amount of TAGs.
†Arbitrary symbols the same as in Table 2.

The chemical structrures of the TAGs produced by the Rhodotorula gracilis at various specific rates of growth were studied by the method of stereospecific analysis (Table 1). The high specificity of sn-2-acyltransferase for unsaturated acids must be noted. The molecular-species and stereotypic compositions of the TAGs were calculated on the basis of the position distribution of the fatty acid residues in the TAGs (Tables 2-5). It was established that for the type composition of the TAGs of Rhodotorula gracilis, at all values of μ a high level of monounsaturated-diunsaturated TAGs (SU_2 = 46.5-53.7%) and also of triunsaturated (U_3 = 21.1-40%) and of disaturated-monounsaturated TAGs (S_2U = 13.8-27.9%) was characteristic. At all values of μ the amount of trisaturated TAGs (S_3) did not exceed 0.4% (Tables 2-5), which is also characteristic for plant oils. The least sensitive to a change in the specific rate of growth ws the amount of the SU_2 type of TAGs, which became possible thanks to the redistribution of the amounts of the individual isomers of this type with a change in μ. With a rise in μ from 0.01 to 0.1 h^{-1} the amount of 1,2-diunsaturated-3-saturated-sn-glycerols (sn-UUS) rose, and that of 1-saturated-2,3-diunsaturated-sn-glycerols (sn-SUU) fell. The amount of monounsaturated-diunsaturated TAGs containing the saturated acyl group in the second position (sn-USU) changed only slightly with a rise in μ (from 0.35 to 0.14%).

At all values of μ the type of disaturated-monounsaturated TAGs (S_2U) was represented mainly by 1,3-disaturated-2-monounsaturated-sn-glycerols (sn-SUS). The total amount of 1,2-disaturated-3-unsaturated (sn-SSU) and 2,3-disaturated-1-unsaturated (sn-USS) TAGs did not exceed 0.73%.

Earlier [1], in a study of the chemical composition of the TAGs of R. gracilis under conditions of batch cultivation we established that in the S_2U-TAGs the dominating species were 2-oleoyldipalmitoyl-sn-glycerol (POP = 14.2%) and 1(3)-stearoyl-2-oleoyl-3(1)-palmitoyl-sn-glycerol (SOP + POS = 10.2%). The same types of TAGs were dominating at $\mu = 0.01\ h^{-1}$, as well. With an increase in the specific rate of growth the second type of TAGs remained among the dominating types, as before, but the amount of the first fell sharply, apparently through a decrease in the amount of palmitic acid in the total fatty acid composition of the TAGs (Table 1).

In the SU_2 type of TAGs, both on batch and on continuous cultivation at all values of μ, 1(3)-palmitoyldioleoyl-sn-glycerol (POO + OOP) and 1(3)-stearoyldioleoyl-sn-glycerol (SOO + OOS) predominated. However, while with an increase in the specific rate of growth the amount of the first type of TAGs fell, the amount of the second rose.

In the U_3 type of TAGs on batch cultivation, trioleoyl-sn-glycerol (OOO) predominated. This species was also predominating at all values of μ studied except $\mu = 0.1$. At $\mu = 0.1\ h^{-1}$, in the U_3 type of TAGs 2-linolenoyldioleoyl-sn-glycerol predominated.

The results of the calculation of the distribution of the TAGs with respect to the number of double bonds in the molecule showed that, at all specific rates of growth of the culture, TAGs with from one to five double bonds in the molecule predominated. With an increase in μ the total amount of the more unsaturated TAGs ($n_e \geq 4$) rose and that of the more saturated (n_e = 0-3) fell. Moreover, the amount of the more unsaturated TAGs ws higher under the conditions of continuous cultivation at all values of μ than that established previously under the conditions of batch cultivation [1]. In view of this, periodic cultivation is more desirable in the production of analogs of oils with a low iodine number (cocoa butter, palm kernel oil).

EXPERIMENTAL

The culture of Rhodotorula gracilis VKM u-335 was obtained from the All-Union Collection of Cultures of Nonpathogenic Microorganisms at IBFM AN SSR [Institute of the Biochemistry and Physiology of Microorganisms of the USSR Academy of Sciences] [4].

The yeast was cultivated in a ANKUM-2M fermenter (IBMF AN SSR, Pushchino) with a working volume of 7.0 liters on a mineral medium of the following composition: glucose, 20.7 g/liter; $(NH_4)_2HPO_4$, 0.87 g/liter; $K_2HPO_4 \cdot 3H_2O$, 0.10 g/liter; KH_2PO_4, 0.08 g/liter; $MgSO_4 \cdot 7H_2O$, 0.05 g/liter; yeast autolyzate, 2 ml/liter; $FeSO_4 \cdot 6H_2O$, 9.95 mg/liter; $CaCl_2 \cdot 6H_2O$, 2.27 mg/liter; $ZnSO_4 \cdot 6H_2O$, 1.31 mg/liter; $CuSO_4 \cdot 5H_2O$, 2.00 mg/liter; $MnSO_4 \cdot 4H_2O$, 0.66 mg/liter; $CoCl_2 \cdot 6H_2O$, 0.20 mg/liter. The process was performed at pH 6.5-6.6, a temperature of 26°C, and a partial presure of oxygen in the medium of 40-60% of saturation. The duration of each regime on cultivation in the chemostat corresponded to a threefold change of the volume of the medium in the fermenter.

The total lipids were extracted by the Bligh–Dyer method [5] from the yeast biomass that had first been frozen to −36°C and had then been mechanically disrupted.

The TAGs were isolated from the total lipids by the countercurrent separation of the neutral and polar lipids in the hexane–87% ethanol (1:1) system recommended by Kates [6] for samples with a high level of TAGs in the total of lipids. The amount of TAGs in the purified fraction of neutral lipids was not less than 95%.

The position distributions of the fatty acids in the TAG molecules were analyzed by Brockerhoff's method [7]. For lipase hydrolysis we used pancreatic lipase type A (Olaine chemical reagents factory). To obtain the phospholipids, as the phosphorylating agent we used phenyl phosphorodichloridate, recommended by Brockerhoff [8]. In the stage of phospholipase hydrolysis we used kufi venom.

The fatty acid composition of the TAGs, of the 2-monoacylglycerols, and of the glycerol lysophosphatides was determined by the GLC method as described in [3].

SUMMARY

1. The position-type composition of yeast TAGs is represented mainly by such types of SUS, SUU, UUS, and UUU, which is also characteristic for plant oils.

2. To obtain unsaturated TAGs it is more desirable to use the continuous cultivation of yeast than batch cultivation.

LITERATURE CITED

1. L. V. Zaitseva, Yu. A. Sultanovich, S. L. Gramolin, and A. P. Nechaev, Khim. Prir. Soedin., 28 (1986).
2. C. Ratledge and M. J. Hall, Biotechnol. Lett., No. 1, 115 (1979).
3. M. V. Rozhdestvenskaya, Yu. A. Sultanovich, and A. P. Nechaev, "Change in the chemical composition of total, the polar and the neutral lipids of a yeast of the genus Rhodotorula as a function of the level of accumulation of the total lipids in the biomass" [in Russian], Moscow (1986). Dep. VNIISÉNTI. No. 304 mb – Dep. 86. 25. 03 (1986).
4. Catalogue of Cultures of the All-Union Collection of Non-pathogenic Microorganisms [in Russian], Nauka, Moscow (1976).
5. E. G. Bligh and W. G. Dyer, Can. J. Biochem. Physiol., 37, p. 911 (1959).
6. M. Kates, Techniques of Lipidology, North Holland, Amsterdam, American Elsevier, New York (1972) [Russian translation], Mir, Moscow (1975), p. 120].
7. H. Brockerhoff, J. Lipid Res., 6, 10 (1965).
8. H. Brockerhoff and N. K. N. Ayengar, Lipids, 14, p. 88 (1979).

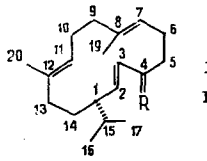
PHOTOCHEMICAL ROUTE TO CEMBRANOIDS CONTAINING A cis-DISUBSTITUTED DOUBLE BOND

A. V. Vorob'ev, S. A. Shevtsov, and V. A. Raldugin

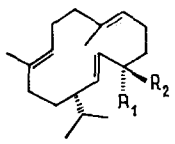
UDC 547.595.9

The 2-cis isomers of the natural diterpenoids isocembrol, 4-epiisocembrol, and isocembrene have been synthesized from a norcembrane ketone by the use of photochemical methods.

A large number of cembrane diterpenoids contain in their molecules a disubstituted double bond in the C-2–C-3 position [1, 2], and in all cases it has the trans (E) configuration. As examples we can give isocembrene (I) [3], isocembrol (II) [4], and 4-epiisocembrol (III) [5]. This paper is devoted to the synthesis of the 2-cis isomers of compounds (I)-(III), their preparation being of interest for the study of their biological activity in view of the fact that some 2-trans-cembranoids exhibit growth-regulating properties [6].



I. R = CH_2
IV. R = O



	R_1	R_2
II.	CH_3	OH
III.	OH	CH_3

As the starting compound for the synthesis of the 2-cis-cembranoids we selected the known [7] ketone (IV). Since cis-trans isomerization is a characteristic photoreaction of α-enols [8] the photolysis of ketone (IV) could be expected to form its 2Z-isomer (ketone (V)). In actual fact, when a pentane solution of ketone (IV) was irradiated with the light of a high-pressure mercury lamp a photoequilibrium mixture of ketones (IV) and (V) (~5:3, according to GLC) was rapidly formed, and these were slowly converted into three other compounds. One of them – the tricyclocembrane ketone (VI) – is described in a separate communication [9].

Ketone (V), like the other photoisomerization products, was isolated by chromatography on silica gel. The cis configuration of its disubstituted double bond was shown by the mag-

Novosibirsk Institute of Organic Chemistry, Siberian Branch, USSR Academy of Sciences.

Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 180-187, March-April, 1988. Original article submittted June 26, 1987; revision submitted September 16. 1987.

0009-3130/88/2402-0150$12.50