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## Abstract

## Full Text

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# NEW TYPES OF NEUTRAL LIPIDS

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As is known, the lipids of animals and plants are characterized by an exceptional diversity in the composition of their fatty acids. At the same time, since Chevreul showed that fats are esters of higher carboxylic acids, the conviction has become firmly established that the sole neutral component of all saponifiable fats is glycerol. In recent years, however, reports have appeared that indirectly indicate the possibility that a number of new nonglyceride esters are present in neutral fats. Thus, Shorland and co-workers, while studying the "triglyceride" fraction of fats of *Sporidesmium bakeri*, noted the absence of a balance between fatty acids and glycerol (^1). Vereshchagin et al. detected, in the chromatography of vegetable oils, nonglyceride lipids of unknown structure with properties close to those of triglycerides (^2). It has been known for a relatively long time that the neutral lipids of mycobacteria contain ethylene glycol (^3, ^4), and recently an ester of meso-2,3-butanediol with trans-vaccenic and palmitoleic acids was isolated from the seeds of the Far Eastern plant *Coix lachryma* (^5, ^6). It should be noted that data of this kind have always been regarded in lipid chemistry as rare anomalies.

Since the available data on the biochemistry of carbohydrate metabolism do not exclude the possibility that, upon cleavage of fructose 1,6-diphosphate, along with glycerol, other polyhydric alcohols may also arise (see, for example, (^7)), on the basis of which complex esters of fatty acids could be formed, the above facts prompted us to carry out a thorough investigation of a series of neutral lipids of animal, plant, and microbial origin. In all the sources studied by us, we indeed found previously unknown lipids, which proved to be esters of fatty acids and various dihydric alcohols.

As is known, the most widespread method for isolating neutral lipids is based on chromatography of the total lipids on silica gel. In this procedure, the triglycerides are eluted with an ether–hexane mixture (5:95), containing, according to various investigators (^8, ^9), only cholesterol esters and free fatty acids as impurities.

In the present work we describe the results of a detailed investigation of the "triglyceride" fractions isolated by the above method from corn and sunflower seeds, mutton fat, cod liver, and

**Table 1**

Gas-chromatographic identification of the diols comprising neutral lipids*
(relative retention volumes)

| Identified substances | Polyethylene glycol succinate, 10%, 150° | Polyethylene glycol succinate, 10%, 150° | Polyethylene glycol succinate, 10%, 150° | Polyethylene glycol succinate, 10%, 150° | Polyethylene glycol succinate, 10%, 150° | Polyethylene glycol succinate, 10%, 150° | High-vacuum grease, 7%, 125° | High-vacuum grease, 7%, 125° | High-vacuum grease, 7%, 125° | High-vacuum grease, 7%, 125° | High-vacuum grease, 7%, 125° | High-vacuum grease, 7%, 125° |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Identified substances | cod liver oil | sunflower oil | mutton fat | cod liver oil | soil yeast** | synthetic samples | corn oil | sunflower oil | mutton fat | cod liver oil | soil yeast** | synthetic samples |
| Diacetate of 2,3-butanediol | 0.057 | 0.057 | 0.062 | — | — | 0.057 | 0.19 | 0.19 | 0.19 | — | — | 0.19 |
| Diacetate of 1,2-propanediol | 0.072 | 0.073 | — | — | — | 0.075 | 0.15 | 0.14 | — | — | — | 0.15 |
| Diacetate of ethylene glycol | 0.088 | 0.088 | — | — | — | 0.093 | 0.11 | 0.12 | — | — | — | 0.12 |
| Diacetate of 1,3-butanediol | 0.13 | 0.12 | 0.11 | 0.11 | — | 0.12 | 0.30 | 0.33 | 0.31 | 0.30 | — | 0.30 |
| Diacetate of 1,3-propanediol | 0.15 | 0.17 | — | 0.15 | 0.15 | — | 0.24 | 0.25 | — | 0.25 | 0.25 | |
| Diacetate of 1,4-butanediol | 0.25 | 0.26 | 0.25 | 0.26 | 0.24 | 0.24 | 0.29 | 0.28 | 0.29 | 0.29 | 0.29 | 0.29 |

* Conditions of gas–liquid chromatography: a Pye chromatograph (England) with a $\beta$-ionization detector, column 1300 × 4 mm, carrier gas—chromosorb. $W$, 60–80 mesh, argon flow rate 50 ml/min, sample size 0.1 $\mu$l, detector voltage

1000 V; the retention volume of glycerol triacetate is 1.00.
** Strain *Lipomyces* sp. No. 40 from the collection of the Department of Soil Microbiology, Moscow State University.

**Table 2**

**Separation of neutral lipids of seeds of Odessa-10 corn* and soil yeasts *Cryptococcus laurentii* No. 26** by preparative thin-layer chromatography*****

| Total neutral lipids, mg | Fraction 1 $R_f$ | Fraction 1 mg | Fraction 1 % | Fraction 2 $R_f$ | Fraction 2 mg | Fraction 2 % | Fraction 3 $R_f$ | Fraction 3 mg | Fraction 3 % | Fraction 4 $R_f$ | Fraction 4 mg | Fraction 4 % | Fraction 5 $R_f$ | Fraction 5 mg | Fraction 5 % | Fraction 6 $R_f$ | Fraction 6 mg | Fraction 6 % | Total fraction mg | Total % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 589 | 0.19 | 54 | 8.9 | 9.4 | 0.82 | 395 | 76.0 | 0.52 | 10.8 | 2.1 | 0.24 | 36.7 | 7.1 | 0.15 | 16.3 | 3.1 | 0.02 | 12.0 | 2.3 | 519.8 | 88.2 |
| (Corn) | | | | | | | | | | | | | | | | | | | | | |
| 525 | 0.19 | 43 | 6.0 | 7.2 | 0.82 | 435 | 86.8 | | | | 0.24 | 12.0 | 2.4 | 0.16 | 18.3 | 3.6 | | | | 501.9 | 95.5 |
| (Soil yeasts) | | | | | | | | – | – | – | | | | | | | – | – | – | | |

* The mixture of neutral lipids was obtained by exhaustive extraction of crushed corn seeds with *n*-hexane.
** A yeast strain from the collection of the Department of Soil Biology, Moscow State University. The total neutral lipids was isolated by extraction of the mycelium with a chloroform–methanol mixture (3:1).
*** For the separation of each 100 mg of mixture, one 18 × 18 cm plate with 18 g of KSK silica gel, 150 mesh, fixed with gypsum, was used; system: heptane + 30% ether. For detection, the edge of the plate was sprayed with 50% sulfuric acid.

soil yeasts. In order to study the alcohol components of the "triglyceride" fractions, they were subjected to alkaline methanolysis ([10]). The methyl esters of fatty acids formed as a result of methanolysis were separated from the free alcohols by chromatography on alumina (activity II) in an ether–hexane (5:95) system. The alcohol fractions eluted with methanol were acetylated ([11]) and analyzed as acetates by gas-liquid chromatography (see Table 1). Comparison of the relative retention volumes of individual components of natural mixtures and synthetic samples showed that glycerol is far from the only polyol entering into the composition of neutral lipids. Along with it, 1,3-butanediol, 1,4-butanediol, and a number of other diols were found in all the "triglyceride" fractions investigated by us (see Table 1).

**Table 3**

**$R_f$ values of fractions of nonglyceride lipids of Odessa-10 corn seeds and soil yeasts *Cryptococcus laurentii* No. 26 during chromatography**

**in a thin layer of silica gel***

| Lipid fractions | System hexane–ether 9:1 $R_f$ | System hexane–ether 9:1 relative $R_f$ | System hexane–benzene 3:7 $R_f$ | System hexane–benzene 3:7 relative $R_f$ |
|---|---|---|---|---|
| Corn | 0.83 | 4.15 | 0.75 | 3.95 |
| Soil yeasts | 0.80 | 4.00 | 0.70 | 3.68 |
| Glycerol trioleate | 0.20 | 1.00 | 0.19 | 1.00 |
| 2-(palmitoyloxy)-butanol-3 | 0.00 | 0.00 | 0.00 | 0.00 |

* Thin-layer chromatography was carried out on 10 × 15 cm plates with 6 g of KSK silica gel, fixed with gypsum ([13]). Detection with phosphomolybdic acid.

As objects for more detailed study we selected Odessa-10 corn seeds and the soil yeast *Cryptococcus laurentii* No. 26. By preparative thin-layer chromatography of the neutral lipids obtained from these sources (Table 2), we were able to isolate, along with the triglyceride fraction (fraction 2), a less polar fraction of nonglyceride lipids (fraction 1), as well as a number of more polar fractions (fractions 3-5)*. In subsequent thin-layer chromatography on silica gel in various systems, fractions 1 and 2 behave as individual substances and can be characterized by $R_f$ values (Table 3). Further study of the fractions of nonglyceride lipids was carried out by treating them with lithium aluminum hydride, acetylating the higher fatty alcohols and polyols thus formed with acetic anhydride, and gas-liquid chromatography of the acetates ([12]). As can be seen from the data in Table 4, fraction 1 of the neutral lipids of corn does not contain glycerides and is a mixture of complex esters of ethylene glycol, 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, and 2,3-butanediol with palmitic, oleic, and linoleic acids. The nonglyceride fraction of the neutral lipids of soil yeasts

* Data on the composition of fractions 3-5 will be the subject of a separate communication.

Table 4

Identification of alcohols formed during aluminohydride treatment of fraction 1 of neutral lipids from seeds of Odessa-10 corn and the soil yeast *Cryptococcus laurentii* No. 26* (relative retention volumes)

| Identified substances | Polyethylene glycol succinate, 10%, 150°: corn | Polyethylene glycol succinate, 10%, 150°: yeasts | Polyethylene glycol succinate, 10%, 150°: synthetic samples | High-vacuum grease, 7%, 125°: corn | High-vacuum grease, 7%, 125°: yeasts | High-vacuum grease, 7%, 125°: synthetic samples |
|---|---|---|---|---|---|---|
| 2,3-Butanediol diacetate | 0.059 | – | 0.057 | 0.19 | – | 0.19 |
| 1,2-Propanediol diacetate | 0.073 | – | 0.075 | 0.15 | – | 0.15 |
| Ethylene glycol diacetate | 0.092 | 0.094 | 0.093 | 0.10 | 0.11 | 0.12 |
| 1,3-Butanediol diacetate | 0.12 | 0.12 | 0.12 | 0.33 | 0.32 | 0.30 |
| 1,3-Propanediol diacetate | 0.15 | 0.15 | 0.15 | 0.24 | 0.24 | 0.25 |
| Cetyl alcohol acetate | 0.67 | 0.66 | 0.67 | – | – | – |
| Stearyl alcohol acetate | – | 1.38 | 1.39 | – | – | – |
| Oleyl alcohol acetate | 1.77 | 1.77 | 1.77 | – | – | – |

| Identified substances | Polyethylene glycol succinate, 10%, 150°: corn | Polyethylene glycol succinate, 10%, 150°: yeasts | Polyethylene glycol succinate, 10%, 150°: synthetic samples | High-vacuum grease, 7%, 125°: corn | High-vacuum grease, 7%, 125°: yeasts | High-vacuum grease, 7%, 125°: synthetic samples |
|---|---|---|---|---|---|---|
| Linoleyl alcohol acetate | 2.12 | 2.12 | 2.12 | — | — | — |

* The gas–liquid chromatography conditions are the same as in Table 1; retention volume of glycerol triacetate, 1.00.

*C. laurentii* No. 26 also represents a mixture of esters of various diols (see Table 4). It was found to contain ethylene glycol, 1,3-propanediol, and 1,3-butanediol, esterified with palmitic, stearic, oleic, and linoleic acids. In parallel with the aluminohydride cleavage, the nonglyceride lipid fraction was subjected to alkaline methanolysis ([10]). Gas-chromatographic analysis of the mixtures of fatty-acid methyl esters obtained as a result of methanolysis fully confirmed the above data on the fatty-acid composition of the nonglyceride fractions (see Table 5). The values of $R_f$ (Table 3) and the IR spectra of the nonglyceride fractions of the neutral lipids of corn and soil yeasts (absence of HO-group bands in the region 3500–3400 $cm^{-1}$) show that they consist exclusively of diesters and do not contain monoacyl derivatives of the named diols.

Table 5

Identification of fatty acids of the nonglyceride fraction of neutral lipids from seeds of Odessa-10 corn and the soil yeast *Cryptococcus laurentii* No. 26* (relative retention volumes)

| Identified esters | Corn | Yeasts | Synthetic samples |
|---|---|---|---|
| Methyl ester of palmitic acid | 0.49 | 0.49 | 0.49 |
| Methyl ester of stearic acid | — | 1.00 | 1.00 |
| Methyl ester of oleic acid | 1.14 | 1.14 | 1.15 |
| Methyl ester of linoleic acid | 1.51 | 1.50 | 1.51 |

* The gas–liquid chromatography conditions are the same as in Table 1; temperature 165°, stationary phase—polyethylene glycol succinate, 10%.

Thus, new types of neutral lipids have been discovered, representing esters of fatty acids with ethylene glycol and various $C_3$- and $C_4$-diols. Apparently, lipids of this type are widely distributed in nature.

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*Note: Figure translations are in progress. See original paper for figures.*