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Occurrence of Arachidonic and Related Acids in the Protozoon *Ochromonas danica*

IT is generally accepted that arachidonic acid is only found in animal lipids. However, similar fatty acids have been reported from several micro-organisms[1–3]. It is therefore of interest to search for the occurrence of arachidonic acid in the lipids of organisms close to the origin of flora and fauna. The composition of fatty acids of the protozoon *Ochromonas danica* has been investigated by us.

This phytoflagellate can be cultivated under photosynthetic conditions and in the dark. The sample used for detailed analysis came from cultures grown in light for 7–10 days on a synthetic medium which did not contain any lipid[4]. The cells were collected by centrifugation; their lipids were extracted by chloroform–methanol, saponified, and the acids esterified[5].

Table 1 gives results of gas-liquid chromatography of the methyl esters[6], but several other acids, some of them not yet identified, were detected in small amounts. The samples did not contain conjugated or *trans* double bonds but alkaline isomerization confirmed the presence of high unsaturation. The tentative identifications by gas-liquid chromatography retention times were confirmed by isolation of the unsaturated fatty esters and determination of their structure.

Isolation proceeded by the following steps: low temperature crystallization removed saturated and oleic acids; liquid–liquid chromatography[7] of the unsaturated fraction yielded essentially two peaks consisting of two and three components; preparative gas-liquid chromatography of 30–40 mgm. samples separated these peak fractions into the individual components, except linolenic and γ-linolenic esters, which were not separated from each other on a preparative scale.

Table 1. MAJOR FATTY ACIDS OF *Ochromonas danica* GROWN IN LIGHT AND CHEMICALLY DEFINED MEDIUM (percentage composition)

Myristic	15	Linolenic	2
Palmitic	10	γ-Linolenic	10
Stearic	3	Arachidic	1
Oleic	8	8,11,14-Eicosatrienoic	5
Linoleic	16	Arachidonic	11
		Docosapentaenoic*	2–4

* Probably 4,7,10,13,16-docosapentaenoic acid.

The positions of the double bonds were determined by ozonolysis and subsequent hydrogenation to aldehydes and aldehyde-esters[8]. These products were identified by gas-liquid chromatography and/or thin-layer chromatography[9] of the 2,4-dinitrophenyl-hydrazones.

Methyl oleate was isolated from the more saturated fraction of the initial crystallization and its structure was verified by the above procedures.

Methyl docosapentaenoate was isolated from another sample of *O. danica* lipids by preparative gas-liquid chromatography of the total esters. Ozonization-hydrogenation showed it to be 4, 7, 10, 13, 16-docosapentaenoate. Gas-liquid chromatography indicated the occurrence of this, or a very similar, acid in the sample described in Table 1.

As with other micro-organisms, the fatty acid composition of *O. danica* varies with the culturing conditions. On defined medium under light the organism produced linolenic and γ-linolenic acids at a ratio of about 1 : 4, and this was not drastically affected when the cultures were grown in the dark. In contrast, their ratio was found reversed when *O. danica* had been grown on a complex medium[10]. However, arachidonic acid represented 10–15 per cent of the total fatty acids at all conditions so far tested.

The discovery of arachidonic together with linoleic, γ-linolenic and 8, 11, 14-eicosatrienoic acids in *O. danica* is of great interest since, in the rat, these latter acids are precursors of arachidonic acid[11]. The occurrence of arachidonic acid in micro-organisms related to *O. danica* is likely. The finding may open a microbiological approach to the study of arachidonic acid metabolism and function.

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[1] Paschke, R. F., and Wheeler, D. H., *J. Amer. Oil Chem. Soc.*, **31**, 81 (1954).
[2] Klenk, E., and Knipprath, W., *Z. Physiol. Chem.*, **317**, 243 (1959).
[3] Wagner, H., Abisch, L., and Bernhard, K., *Helv. Chim. Acta*, **38**, 1536 (1955). Bernhard, K., Abisch, L., and Wagner, H., *Helv. Chim. Acta*, **40**, 1292 (1957).
[4] Aaronson, S., and Scher, S., *J. Protozool.*, **7**, 156 (1960).
[5] Schlenk, H., and Gellerman, J. L., *Anal. Chem.*, **32**, 1412 (1960).
[6] Sand, D. M., and Schlenk, H., *Anal. Chem.*, **33**, 1624 (1961).
[7] Schlenk, H., and Gellerman, J. L., *J. Amer. Oil Chem. Soc.*, **38**, 555 (1961).
[8] Essentially a combination of methods described by Klenk, E., and Brockerhoff, H., *Z. Physiol. Chem.*, **310**, 153 (1958), and by Privett, O. S., and Nickell, C., *J. Amer. Oil Chem. Soc.* (in the press) was used.
[9] Mangold, H. K., *J. Amer. Oil Chem. Soc.*, **38**, 708 (1961).
[10] Haines, T. H., and Block, R. J., *J. Protozool.* (in the press).
[11] Mead, J. F., *Fed. Proc.*, **20**, 952 (1961).

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