Study of the oxidation products of 7-dehydrocholesterol in unsaponified liver fraction of some fishes of the equatorial Atlantic

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Original title: Issedovanie Produktov Okisleniya 7-Degidrokholesterina v neomylyaemoi Fraktsii Pecheni Nekotorykh Ryb Ekvatorial'noi Atlantiki


Translated by the Translation Bureau (GAD)
Multilingual Services Division
Department of the Secretary of State of Canada

Department of the Environment
Fisheries and Marine Service
Halifax Laboratory
Halifax, N.S.
1975

8 pages typescript
TRANSLATED FROM - TRADUCTION DE
Russian

INTO - EN
English

AUTHOR - AUTEUR
Z.A. Vinogradova, R.P. Morozova

TITLE IN ENGLISH - TITRE ANGLAIS
STUDY OF THE OXIDATION PRODUCTS OF 7-DEHYDROCHOLESTEROL IN UNSAPONIFIED LIVER FRACTION OF SOME FISHES OF THE EQUATORIAL ATLANTIC

TITLE IN FOREIGN LANGUAGE (TRANSLITERATE FOREIGN CHARACTERS)
ISSEDOVANIE PRODUKTOV OKISLENIYA 7-DEGIDROKHOLESTERINA V NEOMYLYAEMOI FRAKTSII PECHENI NEKOTORYKH RYB EKVATORIAL'NOI ATLANTIKI

REFERENCE IN FOREIGN LANGUAGE (NAME OF BOOK OR PUBLICATION) IN FULL, TRANSLITERATE FOREIGN CHARACTERS,
Biologiya morya

REFERENCE IN ENGLISH - RÉFÉRENCE EN ANGLAIS
Biology of the sea

PUBLISHER - ÉDITEUR
-

DATE OF PUBLICATION DATE DE PUBLICATION

PLACE OF PUBLICATION LIEU DE PUBLICATION
USSR

1973 - 30

VOLUME

YEAR

ISSUE NO.

NUMÉRO

INDEXED IN

NUMÉROS DES PAGES DANS L'ORIGINAL
pp 140 - 45

8

REQUESTING DEPARTMENT MINISTÈRE-CLIENT
Environment

TRANSLATION BUREAU NO. NOTRE Dossier N°
676461

BRANCH OR DIVISION DIRECTION OU DIVISION
Fisheries Service

TRANSLATOR (INITIALS) TRADUCTEUR (INITIALES)
GAD

PERSON REQUESTING DEMande PAR
Dr. R.G. Ackman

UNEDITED TRANSLATION
For information only

YOUR NUMBER VOTRE Dossier N°

TRADEUCTION NON REVISEE
Information seulement

DATE OF REQUEST DATE DE LA DEMANDE
Dec. 23rd, 1974

STUDY OF THE OXIDATION PRODUCTS OF 7-DEHYDROCHOLESTEROL IN UNSAPONIFIED LIVER FRACTION OF SOME FISHES OF THE EQUATORIAL ATLANTIC

By

Z.A. Vinogradova, R.P. Morozova

Study of the components of unsaponified fractions of tissues of fish is of great theoretical and practical interest, since they include biologically active substances, mainly of a sterol nature.

It was previously shown by V.P. Vendt and colleagues (Vendt, Kuznetsova 1950; Vendt, 1953), and also by Vinogradova and Vendt (1959) that the unsaponified residues of marine invertebrates (molluscs) are characterized by a large amount of 7-dehydrocholesterol and contain in addition substances which have been identified as products of the oxidation of this sterol. The oxides of 7-dehydrocholesterol have been discovered among the components of unsaponified fractions of many organs and tissues of mammals (Vendt, Polyakova, 1955). In time, a specific reaction was developed (Drokova, Vendt, 1959) to reveal products of the oxidation of 7-dehydrocholesterol, which in organochlorine solvents in the presence of traces of concentrated

* Numbers in the right-hand column indicate the corresponding page numbers in the original - Transl.
HNO₃ caused a vivid yellow-green fluorescence. These substances are formed on the oxidation of synthetic 7-dehydrocholesterol under the influence of different oxidation catalysts (Drokova, Vendt, 1959; Vendt, 1961; Morozova, 1969). In addition, under these conditions, oxides of 7-dehydrocholesterol are formed which show a violet fluorescence in organochlorine solvents without the addition of HNO₃.

One of the oxides of 7-dehydrocholesterol was isolated and identified by one of the authors (Morozova, 1969) from unsaponified fractions of sea molluscs (Black Sea mussels) and mammal tissues. Its identity was shown to synthetic oxide obtained from the total of products of oxidized synthetic 7-dehydrocholesterol. Some biological properties were studied (Morozova, 1970). At the present time, study of this substance is being continued.

Of the oxidation products of 7-dehydrocholesterol, the most studied is 5.8-peroxide. This substance was obtained for the first time by Shenk and coworkers (cited in Furst, 1967). The peroxide was discovered in preparations of fish liver by Blondin and Kulkarni (1964). Suggesting a nonphotochemical method of conversion of 7-dehydrocholesterol to vitamin D₃ in fish, the authors consider that 5.8-peroxide can be an intermediate substance in the formation of vitamin D. Other authors, (Hamilton, Casterjon, 1966) suggest that the peroxide is an intermediate substance in the formation of the Δ-5,7-diene system in the process of biosynthesis of cholesterol. In connection with this, it was of interest to study unsaponified residues of fish liver for the purpose of discovering oxidation products of 7-dehydrocholesterol.

We studied unsaponified fractions of liver of fishes caught in the equatorial Atlantic by AtlantNIRO (Kaliningrad) ships: skipjack tuna - Katsuwonus pelamis (Linné), swordfish - Xiphias gladius (Linné), the
shortfin mako - *Isurus oxyrinchus* Rafinesque; blue shark - *Prionaca glauca* (Linneé), thresher shark - *Alopias vulpinus* (Bonuature); the oceanic whitetip shark - *Pterolamiops Longimanus* (Roey). All of the fish species studied are targets of the Soviet fishery in the Equatorial and South Atlantic. We would like to take this opportunity to express our gratitude to E.Z. Samyshev of AtlantNIRO for kindly supplying materials.

Saponification of weighed portions of liver was performed by the usual method. The unsaponified substances were extracted with freshly purified diethyl ether, which was then distilled off in a vacuum. The residue obtained, the weight of which made up 1.5 - 2% of the weighed portion of raw tissue taken, was subjected to further analysis. The basic sterol found in the unsaponified fractions of fish liver studied was cholesterol, with which were present fast sterols in considerable quantity. In addition, the unsaponified fractions also contained in large quantity vitamin A and different fat-soluble pigments. Cholesterol and fast sterols were removed by freezing. Removal of vitamin A and pigments was performed using bentonite. The filtrate was then boiled down in a vacuum, the dry residue was dissolved in chloroform (50 mg of unsaponified residue/1 ml) and subjected to further study.

Separation of components of unsaponified residue was performed using the method of thin-layer chromatography in aluminum oxide with binding gypsum in a benzene-acetone (97:3) system of solvents. The chromatograms were examined in filtered ultraviolet light. The reference spots of the samples tested were developed by a mixture of concentrated \( H_2SO_4 \) and \( H_3PO_4 \) in the presence of \( FeCl_3 \).

As a result of chromatographic separation of the unsaponified residue of liver of the skipjack tuna, 8 clearly defined spots were found on
chromatograms (Fig. 1, a). Our attention was directed to the first three of these. Substance 1, localized in spot with Rf 0.85, was characterized by a yellow fluorescence, substance 2, localized in spot with Rf 0.70 had a violet fluorescence, substance 3 with Rf 0.53 had a vivid yellow-green fluorescence. In the value of Rf and fluorescence, the substances are identical to oxides which were obtained by us from total oxidation products of synthetic 7-dehydrocholesterol.

Spectrophotometric measurements in the ultraviolet zone of the spectrum showed that all three substances did not have typical adsorption peaks (Figure 2).

Similar findings were obtained in the study of unsaponified residues of the liver of sharks and swordfish (Fig. 1, b and c). In addition, in the unsaponified fraction of liver of swordfish, a substance was discovered, localized in spot with Rf 0.18 (Figure 1, d) which was identified by us only from the Rf as the peroxide of 7-dehydrocholesterol, a synthetic preparation of which was obtained in the Department of Photobiochemistry of the Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSSR named after R.I. Yakhimovich.

It should be noted that the research performed is preliminary. However, the study of the chemical and biological properties of the above-mentioned substances is of undeniable interest and is the object of further research.
Fig. 1. Results of chromatographic separation of unsaponified fractions of the liver of tuna (a), shark (b), swordfish (c) – in aluminum oxide in a benzene-acetone (97:3) system of solvents and swordfish (d) – in KSK silica gel in a chloroform-acetone (99:1) system of solvents.
Fig. 2. Ultraviolet adsorption spectra of substances 1, 2, 3 isolated from an unsaponified fraction of the liver of the skipjack tuna.

Key:
1- optical density;
2- wavelength, nm.
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