60°C. The compound (V) was purified on a column with elution by a stepwise gradient of the following solvent systems: chloroform–methanol (9:1), (8:2), (7:3), (1:1), and (2:8), and then with methanol and with chloroform–methanol–water (1:2:0.8). This gave 8 mg of the chromatographically pure substance (V) in the form of an amorphous colorless mass with R_f 0.62 in the chloroform–MeOH–water (64:25:4) system. IR spectrum (cm^{-1}): λ_{max}^{KBr} 2920 (CH_2, CH), 1745 (C=O), 1680, 1525 (NHCO), 1230 (C–O, aldehydogenic bond), 1050 (P–O–C).

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ISOLATION OF PREPARATIONS WITH A HIGH CONTENT OF EICOSAPENTAENOIC ACID FROM THE RED MARINE ALGA *Palmaria stenogona*

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The composition is given of the fatty acids of the red marine alga *Palmaria stenogona*. The amount of eicosapentaenoic acid (EPA) in the alga was 69.9-77.0% of the total fatty acids. The procedure for isolating EPA of 80-90% purity from the alga is described which includes the saponification of the algal lipids, the selective extraction of the unsaponifiable lipids, the isolation of the free fatty acids, and their separation by the method of crystallizing inclusion complexes of the fatty acids with urea.

Eicosapentaenoic acid, a representative of the higher polyenoic acids of the linolenic acid series, is attracting the attention of researchers by its biological activity. It is an essential fatty acid for some species of marine fish and invertebrates and can be used as one of the components of the diet of the animals in mariculture [1, 2]. In higher plants it stimulates the synthesis of phytoalexins possessing fungicidal and bactericidal proper-

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TABLE 1. Composition of the Fatty Acids of the Raw Material for Isolating EPA and of the Accompanying Organisms

Fatty acid, of the total FAs %	Raw material	Accompanying organisms				
	Palmaria stenogona	Scytosiphon lomentaria	Caprellidea	Gammaridea	Gastropoda*	
14:0	3,9	2,6	13,6	0,7	2,0	3,5
14:1	1,1	1,2	—	0,3	1,5	—
16:0	13,6	18,3	16,5	15,1	11,5	7,6
16:1	3,0	2,6	10,4	6,4	4,7	2,6
18:0	0,2	0,6	1,5	6,9	5,0	2,1
18:1	3,4	7,6	13,4	16,8	5,6	3,8
18:2 ω6	0,6	6,1	2,7	3,8	2,4	2,7
18:3 ω3	0,4	6,5	1,5	3,9	3,5	4,6
18:4 ω3	0,5	11,7	1,8	3,3	2,7	10,4
20:4 ω6	3,2	7,1	1,2	4,0	4,7	1,8
20:4 ω3	—	0,9	—	0,7	2,1	2,9
20:5 ω3	69,0	34,9	28,8	35,0	45,6	51,2
22:4	0,9	—	—	0,5	—	0,8
22:5	—	—	—	1,4	4,7	3,6
22:6 ω3	—	—	8,5	5,9	3,9	2,3

*The first column gives the FAs of the whole organisms, and the second the FAs of the egg clusters of gastropod mollusks.

ties [3, 4], which creates the possibility of using preparations of it for protecting plants from diseases. But EPA can give its greatest positive effect in the fight for human health, since it is a potentially important agent for the prophylaxis and treatment of cardiovascular [5, 6], oncological [7], and skin diseases [8], diabetes [9], hypertonia [9], burns [10], and other affections.

Eicosapentoenoic acid is widely distributed in nature [1, 11, 12]. The main sources for its isolation are marine organisms [13-15]. Of biological materials the highest level of it is found in red marine algae [11], in some of which the EPA content reaches 70% [16]. In the present paper we consider methods of isolating EPA from this source.

In the samples that we investigated, the level of EPA varied within wide limits – from 69.0 to 77.0% – and for the experiments we used extracts containing 69.0% of EPA (Table 1). One of the most laborious operations proved to be the sorting of the raw material. We therefore decided to analyze the composition of the fatty acids (FAs) of the accompanying organisms in order to determine how impurities affect the composition of the FAs of the main extract. Among the accompanying organisms there proved to be the brown alga Scytosiphon lomentaria and small crustaceans – skeleton shrimps (Caprellidea), amphipods (Gammaridea), and gastropod mollusks and their egg clusters (Minolia iridescens, Homalopoma sangarense, Epheria turrita). The results of analysis showed that EPA was the main FA in them, the amount of other polyenoic FAs being small, and the impurities themselves making up an insignificant part of the total mass of raw material and therefore being incapable of making appreciable changes in the composition of the fatty acids of the main extract. Subsequently, the stage of sorting the algae was eliminated from the total scheme of the process.

To isolate the FFAs we saponified the lipids of the alga by boiling it in a aqueous alcoholic solution of alkali, and then extracted the FFAs from the mixture obtained [13].

Initially, to obtain EPA from the raw material with a high level of it we used a chromatographic method. The preparation obtained in this way corresponded to the level of the best world standards (EPA content not less than 98%). However, this method is fairly expensive, since it is connected with the use of considerable amounts of silver nitrate and solvents. We have made use of the simpler procedures that are usually employed for the enrichment of polyenoic fatty acids in mixtures of acids in working with larger amounts of substances than can be separated chromatographically. The method of crystallizing inclusion complexes of the FAs with urea [17] proved to be convenient for these purposes, since, in the first place, it is technically simple and in the second place, in view of the fact that 25% of the total FAs of the raw material consists of saturated and monoenoic acids, one could expect that this method would permit a product to be obtained with such a level of EPA that in the isolation of this acid from other sources is achieved only by chromatography [13, 14].

TABLE 2. Conditions for the Isolation of EPA from the Alga Palmaria stenogona by the Crystallization of Inclusion Complexes of the Fatty Acids with Urea

Form of FA mixture crystallized	EPA, % of the total FAs		Yield of desired product, % on theoretical	Weight ratio of mixture to be crystallized and urea
	in the initial mixture	in the final product		
Methyl esters	69,0	84,2	27,2	1:3
FFAs	69,0	88,0	50,2	1:3
FFAs	69,0	82,4	82,7	1:2
FFAs	69,0	72,3	—	1:1

For crystallization with urea we used both the FFAs [13, 18] and also fatty acid esters [19]. A trial showed that to obtain concentrates of EPA from the alga it is more convenient to take the FFAs for crystallization – the final product differed little in purity from the product obtained on the crystallization of the methyl esters, while the yield was twice as great (Table 2).

In the choice of the proportions of urea and the FFA mixture we were interested in those ratios at which it was possible for as little EPA as possible to be present in the crystals of the complex but which would be sufficient for binding the saturated and monoenoic acids that are present in the mixture of fatty acids of P. stenogona in amounts of up to 25% of the total. We tested three weight ratios of urea to FFAs – 3:1, 2:1, and 1:1 (see Table 2). At the highest ratio the purest product was obtained (with an EPA content of 88.0%), but almost half the EPA present in the initial mixture of acids was lost with the precipitate. At a ratio of 2:1, only 17.0% of the EPA proved to be present in the crystals, and the remainder could be isolated from the filtrate, the purity of the final product being 82.4%. At a 1:1 ratio there was practically no enrichment of the primary raw material with EPA.

In the choice of conditions for obtaining the EPA concentrate it was necessary to start from concrete problems. A urea:FFA ratio of 3:1 was the most convenient for obtaining concentrates with a high level of EPA. We have used a ratio of 2:1 for obtaining concentrates which were then purified chromatographically. The latter procedure permitted a product containing more than 99.0% of EPA to be obtained from the alga P. stenogona.

EXPERIMENTAL

Specimens of the algae and the accompanying organisms were collected in Vityaz Bay (Posyet Bay, Sea of Japan) in the winter months. The algae (1 kg) were washed with sea water and fresh water, dried in the air, covered with 1 liter of ethanol and a solution of 30 g of caustic soda in 100 ml of water, and heated in the water bath for 2 h. The liquid was poured off and was used for the subsequent treatment of two new portions of algae. The algae that had been extracted last were additionally boiled in 0.5 liter of ethanol and a solution of 10 g of caustic soda in 0.5 liter of water. All the alcoholic extracts were combined, and the unsaponifiable substances were eliminated with 1 liter of petroleum ether. The remaining concentrate was acidified with 10% sulfuric acid solution, the pH being brought to 1, and after phase separation the fraction containing the FFAs was collected. The extraction of the FFAs was repeated with two 200-ml portions of petroleum ether. The combined extracts were evaporated and the concentrate was diluted with hexane.

The extracts of the accompanying organisms were obtained by the same procedure as was used for the isolation of the FFAs from the alga P. stenogona.

The method described by Christie [19] was used for the methylation of the FFAs.

The crystallization of the FFAs or their methyl esters with urea was carried out in methanol at various weight ratios of the mixture to be fractionated and urea (1:3, 1:2, and 1:1). The mixture of fatty acids or their esters was added to a solution of urea in methanol, and the resulting mixture was stirred and left at room temperature for 3 h and then at −10°C for 15 h. The crystals that had formed were separated off on a Schott filter and were washed with a saturated solution of urea in methanol. The combined filtrates were con-

centrated to half their initial volume and were acidified with a 1% solution of hydrochloric acid, and the polyenoic fatty acids or their esters were extracted first with hexane and then with diethyl ether.

The method of column chromatography on silica gel impregnated with silver nitrate [15] was used to obtain highly purified EPA.

The fatty acids were analyzed by GLC on a Shimadzu GC-5 chromatograph (Japan) with a flame-ionization detector. Glass column 0.3 × 300 cm filled with 3% of Silar 5CP deposited on Chromaton N 0.100-0.125 mm. The identification and quantitative calculations were carried out by methods described previously [20].

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