

Steroids. R<sub>f</sub> 0.40, 0.16, 0.05. They gave a characteristic coloration on treatment with sulfuric acid. The component with R<sub>f</sub> 0.16 migrated together with  $\beta$ -sitosterol (solvent system 8).

Chlorophyll  $\alpha$ . UV spectrum,  $\lambda_{\text{max}}^{\text{acetate}}$ , nm: 410 max, 422 w, 504 w, 535 w, 570 w, 610 m, 656-668 [5].

#### SUMMARY

A comparative study of some lipids of four forms of common sea buckthorn has shown that:

1. All four forms differ sharply in the composition of the fatty acids of the triacylglycerols from the leaves.

2. The sea buckthorn of form 1 differs from the other three by the fact that in the triacylglycerides of the oil of its fruit and leaves the law of the esterification of the positions 2 by unsaturated fatty acids is infringed.

3. The composition of the free fatty acids of the oil of the fruit differs quantitatively from that of the fatty acids of the triacylglycerols of the oils of the fruit and is close to the composition of the fatty acids of the triacylglycerols of the leaves through the presence of fatty acids with 22-25 carbon atoms.

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#### UBIQUINONES OF MARINE INVERTEBRATES

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UDC 547.567:592(26)

The quantitative and qualitative compositions of the ubiquinones of 28 species of marine invertebrates representing five main types have been studied. The amount of ubiquinones did not exceed 5  $\mu$ g per 1 g dry weight. For all species the main component is Q<sub>10</sub>, except for ascidians, which produce only Q<sub>9</sub>.

The distribution of ubiquinones (Q<sub>n</sub>) in living organisms is generally correlated with the aerobic metabolism of their tissues. In higher animals and plants Q<sub>9</sub> and, mainly, Q<sub>10</sub> are found. Microorganisms are capable of synthesizing all natural ubiquinone homologs but they contain mainly Q<sub>6</sub>-Q<sub>9</sub> [1].

Possessing a very broad spectrum of therapeutic action, ubiquinones are finding ever increasing use in medical practice. In view of this, intensive searches are being carried out for natural sources and synthetic routes for their production [2].

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Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from *Khimiya Prirodnikh Soedinenii*, No. 6, pp. 715-718, November-December, 1981. Original article submitted April 20, 1981.

TABLE 1. Amount and Composition of the Ubiquinones of Marine Invertebrates

Type	Class	Species	Q <sub>n</sub> *	Amount of Q <sub>n</sub> †
Coelenterata	Anthozoa	Alcionidae sp. 1	10	1.2
		Alcionidae sp. 2	10	1.3
Annelida	Polichaeta	Eurithoe complanata	10	0.8
Mollusca	Gastropoda	Cipreae tigris	10	2.3
		Trochus niloticus	10	3.2
		Haliotis asinina	10	1.9
		Conus tulipa	10	1.9
		Strombus luhuanus	10	1.5
		Aplisia dactylomela	10	1.4
		Conus litteratus	10.9	1.3
Echinodermata	Bivalvia	Tridacna maxima	10	4.0
	Crinoidea	Camanthus bennetti	10	1.8
		Camantheria briareus	10	2.2
		Himerometra robustipinna	10	1.7
	Asteroidea	Aconthaster planci	10	2.0
		Letasterias fusca	10	2.5
		Patiria pectinifera	10	3.3
		Aphelasterias japonica	10	2.0
		Asterias amurensis	10	2.0
		Lysastrosoma anthosicta	10	3.9
		Evasterias echinosoma	10	2.9
		Distolasterias nipon	10	
	Echinoidea	Diadema setosum	10	5.6
	Holothuroidea	Holothuria atra	10	1.9
		Cucumaria fraudatrix	10	2.7
Chordata	Ascidiae	Ascidiae sp.	9	1.2
		Halicynthia auranthina	9	2.2
		Halicynthia roretzi	9	1.8

\*Number of isoprene units in the side chain of the ubiquinone.

†Amount of ubiquinone, µg per 1 g crude weight.

While broad information has accumulated on the amount and composition of ubiquinones of microorganisms, terrestrial animals, and plants, there is practically no such information on marine invertebrates. At the beginning of our investigation only a few publications devoted to the study of the qualitative composition of the ubiquinones were known [1, 3].

The aim of the present investigation was to determine the quantitative content and to establish the qualitative composition of the ubiquinones from the representatives of several types of marine invertebrates. Use was made of the method of alkaline saponification, which permits the complete extraction of ubiquinones present in animal tissues [4]. Control experiments on the extraction of Q<sub>10</sub> from a crude and a freeze-dried preparation of ox heart gave ubiquinone contents of 68 ± 8 and 260 ± 15 µg calculated to 1 g of the preparation, respectively. These amounts agree closely with literature figures [1]. The recovery of a preparation of a standard sample of Q<sub>9</sub> added before saponification was 95%. As standard samples of ubiquinones we used Q<sub>10</sub> isolated from ox heart, and also preparations of Q<sub>6</sub> and Q<sub>9</sub>.\*

As can be seen from the table, only Q<sub>10</sub> was found in all the invertebrates studied. The sole exception consisted of preparations of ascidians, containing only Q<sub>9</sub>. Q<sub>9</sub> was also detected in the mollusk *Conus litteratus*, but its amount was an order of magnitude lower than that of the main component — Q<sub>10</sub>. It is possible that the lower homologs are present in trace amounts also in the other animals that we studied, but the method which we used does not permit their detection. Similar results on the qualitative composition of the ubiquinones have been obtained by British scientists [3]. They investigated 14 species of invertebrates belonging to various types. They all contained Q<sub>10</sub>, with the exception of the mollusk *Nucella lapillus*, in which Q<sub>9</sub> was found.

\*The preparations were kindly supplied by G. I. Samokhvalov (All-Union Scientific-Research Vitamin Institute).

The amount of ubiquinone in the invertebrates that we studied was 1.2-3  $\mu\text{g/g}$ ; a higher amount was found in the mollusk *Tridacna maxima* and in the sea urchin *Diadema setosum*. The results given on the quantitative amount and qualitative composition of the ubiquinones in marine invertebrates agree with the results obtained recently by Norwegian scientists [5].

#### EXPERIMENTAL

Animals belonging to five types were collected during the seventh tropical expedition of the scientific-research ship "Professor Bogrov" (1979) and at the marine experimental station of the Pacific Ocean Institute of Bioorganic Chemistry (Posyet Bay, Sea of Japan).

The previously comminuted fresh tissue of an animal (200 g) was homogenized for 3 min. The saponification of the homogenate in an aqueous ethanolic solution of caustic potash in the presence of pyrogallol in an atmosphere of argon and the extraction of the unsaponifiable lipids with hexane was carried out by the method described by Crane and Barr [6]. The subsequent separation and determination of the amounts of ubiquinones were also carried out by methods described in this source. The dried extract was dissolved in 100 ml of hexane, poured into a tube in an atmosphere of argon, and stored at  $-5^{\circ}\text{C}$ . The ubiquinones were isolated from the unsaponifiable fraction of the lipids by TLC on Chemapol silica gel with benzene as eluent. An aliquot, usually 5 ml of the hexane solution after it had been evaporated to 25 ml, was separated on a  $20 \times 20$  cm plate. To detect the ubiquinones we used an aqueous solution of reduced methylene blue. The silica gel zone containing the ubiquinones was transferred quantitatively to a filter and they were extracted with anhydrous ethanol. Then the ubiquinones were separated by TLC on a Silufol plate impregnated with 5% of paraffin oil. The system for elution was propan-1-ol-water (4:1) saturated with paraffin oil. The position of the ubiquinone spot on the plate was determined with the aid of reduced methylene blue and was compared with the position of the spots of standard ubiquinones. The ubiquinones were extracted quantitatively with anhydrous ethanol and transferred to a 10-ml measuring flask. They were determined quantitatively by a spectrophotometric method on a VSU2-P instrument. An ethanolic solution of an ubiquinone was reduced directly in the measuring cell with a few small crystals of sodium tetrahydroborate. For calculation we used the difference in the extinctions of the oxidized ( $E_{\text{ox}}$ ) and reduced ( $E_{\text{r}}$ ) forms of ubiquinone at 275 nm by means of the formula

$$\frac{E_{\text{ox}} - E_{\text{r}}}{\Delta \epsilon_{\text{ox-r}}} = \text{micromoles of } Q_n \text{ in 1 ml of solution,}$$

where  $\Delta \epsilon_{\text{ox-r}}$  is the difference in the extinctions of the oxidized and reduced form for 1 micromole of ethanolic solution of ubiquinone in a cell with a layer thickness of 1 cm.

#### SUMMARY

The amounts of ubiquinones in the representative of five types of marine invertebrates that were studied were at the level of 1.2-3  $\mu\text{g/g}$ , not exceeding 6  $\mu\text{g}$  per 1 g dry weight.  $Q_{10}$  was the main component for all types with the exception of ascidians, which produce only  $Q_9$ .

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