CAROTENOIDS OF THE ASCIDIAN *Halocynthia aurantium*

N. M. Rebachuk, O. B. Maksimov,
L. S. Boguslavskaya, and S. A. Fedoreev

UDC 547.979.8:594.933:542.943.8

It has been established that the main components of the carotenoid fraction of an extract of *Halocynthia aurantium* are astaxanthin and diatoxanthin, the latter possessing a weak antioxidant activity. No prenylated hydroquinones, which are characteristic for colonial species of ascidians were detected in the four species of solitary ascidians from the Sea of Japan investigated.

Interest in the study of the chemical composition of ascidians (Tunicata) is due to the wide food use of individual species of these marine invertebrates (solitary forms) in the countries of the Far Eastern region.

Substances with various biological activities and, in particular, prenylated hydroquinones possessing antitumoral activity have been found in certain colonial ascidians [1, 2]; we have no such information in the literature for solitary species of ascidians.

In this connection, we have tested for the presence of these aromatic metabolites in four species of solitary ascidians living in the Sea of Japan: *Halocynthia aurantium*, *Halocynthia roretzi*, *Cnemidocarpa heterotentaculata*, and *Styela clava*.

The repeated analysis of ethanolic and acetonic extracts of the animals in the course of two annual seasons showed the absence of the compounds sought.

In the process of investigation, from the tunic of *Halocynthia aurantium* (wastes in the use of these animals for food) we isolated a series of carotenoids which may find use as food dyes. The structures of the two main components have been established and the parameters characterizing their antiradical and antioxidant activities have been determined.

With a total carotenoid content of 0.3% of the weight of the dry tunic, the yields of these components (fractions 2 and 4, Table 1) amounted to 45% and 30% of the total, respectively.

The spectral characteristics of the substance of fraction 2 corresponded to those given in the literature for diatoxanthin [4, 5], a carotenoid diol containing one triple bond.

The melting point and spectral characteristics of fraction 4 coincided completely with those of astaxanthin [3]. The product of the reduction of an ethanolic solution of this fraction by $NaBH_4$ showed λ_{max} 451 (426 sh., 478) nm, which corresponds to the spectral absorption of crustaxanthin, a tetrahydric alcohol obtained by the reduction of astaxanthin. The results given unambiguously show that the substance of fraction 4 was astaxanthin.

To characterize the antiradical activities of the carotenoids investigated, on the low-molecular-weight model of initiated oxidation of cumene [6] we determined the kinetic constants K_7, K_{10}/K_8, and K_7/K_8 and the values of the stoichiometric factors f (Table 2).

The constants of the reaction with free radicals (K_7) for both compounds were less than the corresponding constant for ubiquinone ($7 \cdot 10^4$) and tocopherol ($2.6 \cdot 10^6$) [7], but they were close to those for β-carotene ($1.75 \cdot 10^4$) [8] and for Ionol ($2.4 \cdot 10^4$) obtained in the oxidation of ethylbenzene.

Table 2 shows that the carotenoids investigated should be weak antioxidants since the radicals formed from them are capable of propagating a chain of oxidation by a reaction with hydroperoxides and also with the oxidation substrate RH (reactions 7 and 10*).

*The generally adopted numbering of chain oxidation reactions [6] is used.

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 431-433, July-August, 1984. Original article submitted June 15, 1983.

0009-3130/84/2004-0407$08.50

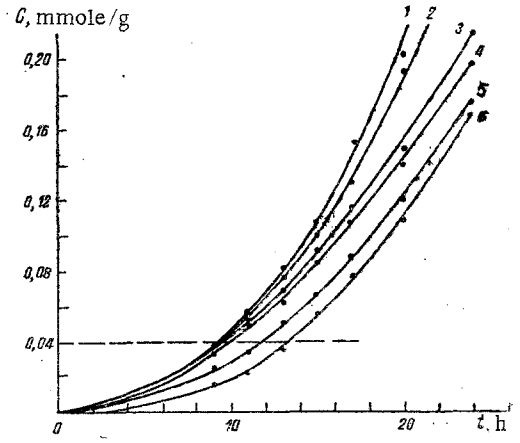

Fig. 1. Kinetic curves of the accumulation of hydroperoxide in the oxidation of methyl oleate (3), and of methyl oleate in the presence of astaxanthin [4) 0.1 mg/ml; 1) 4 mg/ml], of diatoxanthin [5) 0.1 mg/ml, 6) 4 mg/ml], and of β-carotene [2) 4 mg/ml]. C is the concentration of hydroperoxides.

TABLE 1. TLC of an Acetone Extract of *Halocynthia aurantium**

Zone	R_f	Coloration
1	0,22	Orange
2	0,30	Dark yellow
3	0,36	Light orange
4	0,41	Dark orange
5	0,45	Lilac
6	0,48	Pink
7	0,52	Yellow
8	0,54	Pink
9	0,56	Orange
10	0,58	Pink
11	0,60	Light yellow

*Silufol; benzene—acetone (10:2).

TABLE 2. Characteristics of the Antiradical Activities of the Carotenoids

Carotenoid	K_7, liter/mole·sec	K_{10}/K_8	K_7/K_8	f
Astaxanthin	$0,57 \cdot 10^4$	$2,5 \cdot 10^{-9}$	$0,3 \cdot 10^{-5}$	4
Diatoxanthin	$1,40 \cdot 10^4$	$1,0 \cdot 10^{-8}$	$0,5 \cdot 10^{-5}$	4

The antioxidant activities of the preparations were checked on a methyl oleate model at 55°C [9]. Figure 1 gives kinetic curves of the accumulation of peroxides in the presence of astaxanthin, diatoxanthin, and β-carotene.

Astaxanthin, like β-carotene, possesses practically no antioxidant activity, and at high degrees of oxidation actually accelerates the process. Somewhat different results were obtained for diatoxanthin. It is a weak antioxidant the relative activity of which in comparison with that of Ionol (τ for diatoxanthin/τ for Ionol) is 0.02. The dependence of the induction period on the concentration is not linear for diatoxanthin, which shows an influence of the products of its transformation on the oxidation of methyl oleate.

EXPERIMENTAL

The animals (*Halocynthia aurantium*) were collected in June, 1981, in Peter the Great Bay. An alkaline extract of the tunic of the ascidian when separated on Silufol plates showed the presence of numerous carotenoid spots (Table 1); it was separated on a column of silica gel L (Czechoslovakia) in a benzene-acetone gradient with the percentage of acetone rising from 0 to 100%. The fractions corresponding to zones 2 and 4 were investigated in detail. Absorption spectra were obtained on Cary 219 (Varian) instrument, IR spectra on a Specord 75-IR, PMR spectra on a Bruker HX-90E, and mass spectra on a LKB-9000S instrument.

The substance of fraction 2 was obtained in the form of yellow-orange plates with mp 198°C (hexane–chloroform). Absorption spectrum, λ_{max}, nm: 447, 425 sh., 476 (hexane); 452, 427 sh., 480 (ethanol); 461, 435 sh., 489 (chloroform). IR spectrum, $\nu_{max}^{CHCl_3}$, cm^{-1}: 3605 (OH), 2900, 2940, 2900 (CH), 2172 (C≡C), 1566 (C=C), 1450 (CH_2), 1360 (gem. CH_3). Mass spectrum (m/z): 566 (M^+), 564, 548, 474. PMR spectrum (ppm): 1.07 (6 H, Me, 1', 1'), 1.14 1.20 (6 H, Me, 1.1), 1.74 (3 H, Me, 5'), 1.92 (3 H, Me, 5), 1.96 (9 H, Me, 13, 13', 9'), 1.99 (3 H, Me, 9), 3.99 (2 H, OH, 3, 3').

The substance of fraction 4 crystallized in the form of dark violet needles with mp 214°C (methanol). In the visible part of the spectrum there was a single peak with λ_{max} (nm) 470 (hexane), 477 (ethanol), 478 (acetone), 483 (chloroform). IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3490 (OH); 3040, 2930, 2870 (CH); 1650 (C=O); 1550 (C=C); 1550 (C=C). Mass spectrum (m/z); 596 (M^+), 594, 580, 564, 517, 504, 502. PMR spectrum (ppm): 1.21; 1.32 (12 H, gem, Me, 1, 1'), 1.94 (6 H, Me, 5, 5'), 2.00 (12 H, Me, 9, 13, 9', 13'), 3.66 (2 H, OH, C-3,3').

SUMMARY

In the species of solitary ascidians investigated none of the prenylated hydroquinones that are characteristic for colonial species of these animals were detected. The main components of a carotenoid fraction of an extract of *Halocynthia aurantium* were astaxanthin and diatoxanthin. The latter possessed weak antioxidant activity.

LITERATURE CITED

1. B. M. Howard, K. Clarkson, and R. L. Bernstein, Tetrahedron Lett., 49, 4449 (1979).
2. K. L. Rinnehart, J. B. Gloer, R. G. Hughes, H. E. Renis, J. P. McGovern, E. B. Smynenberg, D. A. Stringfellow, S. L. Kuentzel, and L. H. Li, Science, 212, 933 (1981).
3. A. Khare, G. R. Moss, B. C. L. Weedon, and A. D. Matthews, Comp. Biochem. Phys., 45, 971 (1973).
4. F. H. Foppen, Chromatogr. Rev., 14, 133 (1971).
5. T. Bjornland, Phytochemistry, 21, 1715 (1982).
6. V. V. Fedorova, and V. V. Kharitonov, Zh. Fiz. Khim., 47, 2425 (1973).
7. Yu. A. Zaslavskii, N. G. Khramova, I. G. Akoev, S. F. Terekhova, and E. B. Burlakova, Biofizika, 2, 359 (1977).
8. O. T. Kasaikina, Z. S. Kartasheva, and A. B. Gagarina, Izv. Akad. Nauk SSSR, Ser. Khim., No. 3, 356 (1981).
9. E. B. Burlakova, A. V. Alesenko, E. M. Molochkina, N. P. Pal'mina, and N. G. Khrapova, Bio-antioxidants in Radiation Damage and Malignant Growth [in Russian], Moscow (1975), p. 11.