Journal of Natural Products
Vol. 48, No. 4, pp. 606-613, Jul-Aug 1985

CAROTENOIDS OF TUNICATES, III. THE STRUCTURAL ELUCIDATION OF TWO NEW MARINE CAROTENOIDS, AMAROUCIAXANTHIN A AND B[1,2]

TAKAO MATSUNO,* MASAHIRO OOKUBO, and TADAAKI KOMORI

Department of Natural Products Research, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan

ABSTRACT.—The structure of two new marine carotenoids, isolated from the tunicate, *Amaroucium pliciferum*, and designated as amarouciaxanthin A and B, were determined on the basis of chemical and spectral data. Amarouciaxanthin A was determined as (6*S*, 3′*S*, 5′*R*, 6′*R*)-6,3′,5′-trihydroxy-4,5,6′,7′-tetradehydro-7,8,5′,6′-tetrahydro-β,β-carotene-3,8-dione, and Amarouciaxanthin B was determined as (6*S*,3′*R*)-6,3′-dihydroxy-4,5,7′,8′- tetradehydro-7,8-dihydro-β,β-carotene-3,8-dione.

These two compounds are also presumed to be new metabolic products of a typical marine carotenoid, fucoxanthin.

In 1958, Nishibori (1) reported that a free xanthophyll, having absorption maxima at 479 and 503 nm in CS_2, was first isolated from the sea squirt *Halocynthia roretzi*. Subsequently, in the course of our comparative biochemical studies of marine carotenoids and of studies on the metabolism of fucoxanthin in marine animals, we found two new carotenoids, halocynthiaxanthin (**1**) (2) and mytiloxanthinone (**2**) (3) from the sea squirt, *H. roretzi*. Further extensive research for carotenoids of tunicates led to the isolation of two new carotenoids from *Amaroucium pliciferum* Redikorzev. These carotenoids were designated as amarouciaxanthin A and B, referring to *Amaroucium* from which the carotenoids were isolated. It is very interesting from a biosynthetic point of view that *A. pliciferum* possesses high contents of amarouciaxanthin A (**3**) and B (**4**) (See Results section).

In this paper we describe the structural elucidation of amarouciaxanthin A (**3**) and B (**4**)[3], and their biological characteristics.

RESULTS

Amarouciaxanthin A (**3**) (7% of total carotenoids) was obtained as reddish needles (from petroleum ether) to yield 2 mg from 6 kg of specimens, $C_{40}H_{54}O_5$ (M^+, 614) uv-visible λ max 230, 269, and 455~460 nm (Et_2O). The uv (230 nm) and ir [ν max (KBr) 1660 cm^{-1}] suggested the presence of a conjugated enone chromophore, and an allenic end group was proved by ir (1928 cm^{-1}) and ^1H nmr (δ 6.00 (1H, s)). On reduction with sodium borohydride, **3** gave a product with visible maxima at 399, 422, and 449 nm (in Et_2O), in good agreement with those of fucoxanthol (**5**) (4). This suggested that the reduction of a single in-chain conjugated carbonyl of **3** had led to the same chromophore as fucoxanthol (**5**). In the ms of **3**, the fragment peaks at *m/z* 596 (M^+-18), 578 (M^+-18-18), and 560 (M^+-18-18-18) indicated the presence of three hydroxyls, one of which was acetylated with Ac_2O/pyridine. These facts suggested the presence of one primary or secondary hydroxyl and two tertiary hydroxyl groups. The

[1]This work was presented at the 7th International Symposium on Carotenoids, München, 27 August 1984.

[2]Taken in part from the Ph.D. Thesis of Masahiro Ookubo Kyoto Pharmaceutical University, 23 January 1984.

[3]During the preparation of this manuscript, C. Belaud and M. Guyot reported the isolation and structural elucidation of sidnyaxanthin which seems to be identical with amarouciaxanthin B; see *Tetrahedron Lett.*, **25**, 3087 (1984).

prominent peak at *m/z* 462.3114 (calcd. for $C_{31}H_{42}O_3$: 462.3123) was attributed to cleavage of the C6-C7 bond. The presence of the 6-hydroxy-ε-caroten-3-one moiety in **3** was suggested by the following spectroscopic data; the proton signals at δ 1.05 (3H, s, 16-Me), 1.08 (3H, s, 17-Me), 1.89 (3H, s, 18-Me), 2.35 and 2.48 (2H, ABq, J=18 Hz, 2-CH_2), and 5.81 (1H, s, 4-CH=). In addition, the presence of the 3′,5′-dihydroxy-6′,7′-didehydro-5′,6′-dihydro-β-carotene moiety in **3** was indicated from the proton signals at δ 1.08 (3H, s, 16′-Me), 1.34 (6H, s, 17′- and 18′-Me), 1.5~2.5 (4H, m, overlapped another protons, 2′- and 4′-CH_2), and 4.20 (1H, m, 3′-CH) in the ^{1}H nmr.

The treatment of **3** with 10% KOH/MeOH gave 6-oxo-isophorone (**6**) and paracentrone (**7**) (5) (Figure 1). The spectral data (uv, and mass) of both compounds were each consistent with those of authentic samples. These facts support the validity of the structure for amarouciaxanthin A (**3**) as proposed (Figure 1).

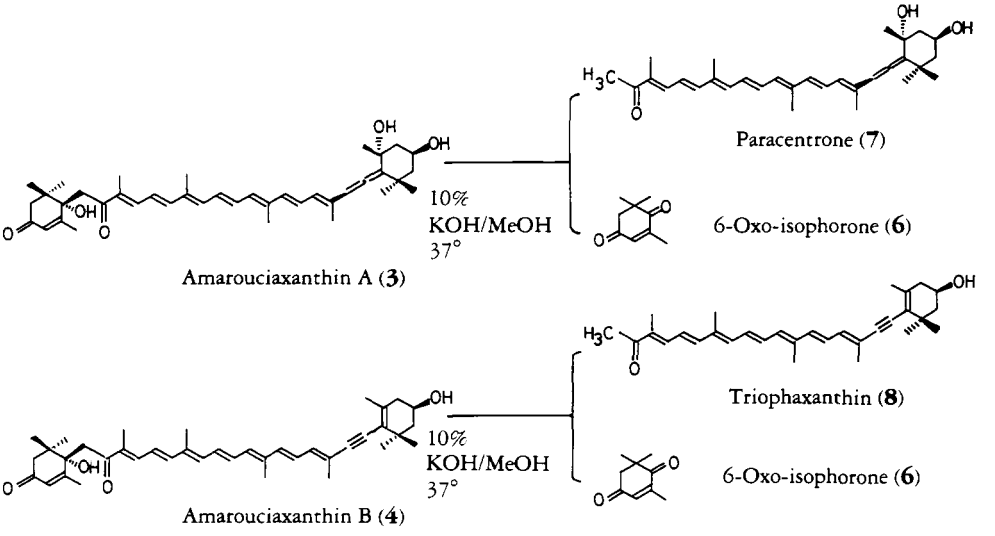
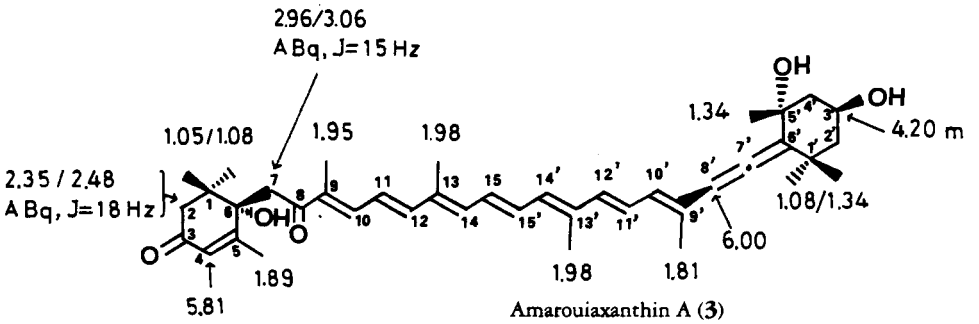


FIGURE 1. Treatment of amarouciaxanthin A (**3**) and (B) (**4**) with 10% KOH/MeOH.

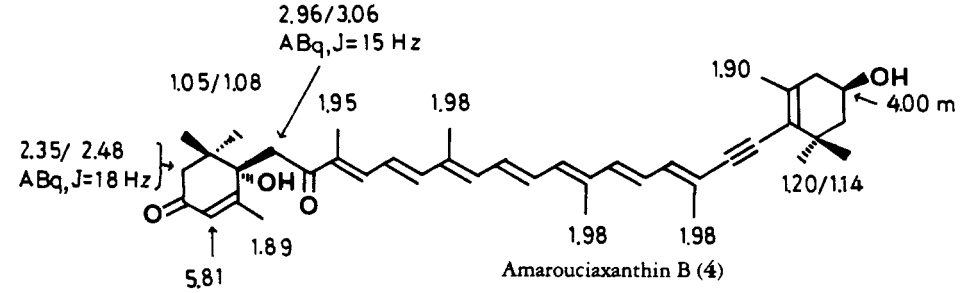
Amarouciaxanthin B (**4**) (43% of total carotenoids, principal carotenoid), was isolated as reddish needles to yield 10 mg from 6 kg of specimens, $C_{40}H_{52}O_4$ (M^+, 596), uv-visible max 230, 281, and 463 nm. The uv (230 nm) and the ir (1660 cm^{-1}) suggested the presence of a conjugated enone. The ir of **4** resembles that of **3** except for 2165 cm^{-1}. The ^{13}C nmr shows also the presence of a disubstituted acetylenic group [89.5 (s) and 98.6 (s)]. On reduction with sodium borohydride, **4** afforded a product with visible maxima at 405(sh), 428, and 453 nm, whose maxima were identical with the compound derived from halocynthiaxanthin (**1**) in the same manner. This suggested the reduction of a single in-chain conjugated carbonyl. In the ms of **4**, the fragment peaks at *m/z* 578 (M^+-18) and 560 (M^+-18-18) indicated the presence of two hydroxyls. Acetylation (Ac_2O/pyridine) of **4** gave a monoacetate. These facts indicated the presence of one primary or secondary and one tertiary hydroxyl. The prominent peak at *m/z* 444.3033 (calcd. for $C_{31}H_{40}O_2$: 444.3018) was based on cleavage of the C6-C7 bond. ^{1}H-nmr analysis of **4**, together with the ^{13}C nmr, revealed the presence of a 6-hydroxy-ε-caroten-3-one moiety, as observed in **3**, and 3′-hydroxy-7′,8′-didehydro-β-caroten (δ 1.14 (3H, s, 16′-Me), 1.20 (3H, s, 17′-Me), 1.90 (3H, s, 18′-Me), 1.48 (1H, dd, J=11.5 and 12 Hz, 2′-CH_{ax}), 1.82 (1H, dd, J=1.5 and 12 Hz, 2′-CH_{eq}), 2.12 (1H, dd, J=10 and 17 Hz, 4′-CH_{ax}), 2.45 (1H, dd, J=7 and 17 Hz, 4′-CH_{eq}), and 4.00 (1H, m, 3′-CH) in the ^{1}H nmr; 22.5 (q, 18′-Me), 28.8 (q, 16′-

Me), 30.6 (q, 17′-Me), 36.6 (s, 1′-C-), 41.5 (t, 4′-CH_2-), 46.7 (t, 2′-CH_2-), 64.8 (d, 3′-CH-), 124.2 (s, 6′-C=), and 138.4 (s, 5′-C=) in the ^{13}C nmr. In order to support the validity of the assignment of each signal in the ^1H nmr and ^{13}C nmr, saponification of **4** was conducted to yield 6-oxo-isophorone (**6**) and triophaxanthin (**8**) (6) (Figure 1).

From these results, the structure of **4** was proposed as shown in Figure 1. In addition, diatoxanthin (**9**) (7), alloxanthin (**10**) (8), diadinochrome (**11**) (9), mytiloxanthin (**12**) (10), fucoxanthin (**13**) (4), halocynthiaxanthin (**1**), and fucoxanthinol (**14**) (4) were isolated together with **3** and **4** as described in the Experimental section.



Amarouiaxanthin A (**3**)



Amarouciaxanthin B (**4**)

DISCUSSION

The stereochemistry and the biological characterization of amarouciaxanthin A (**3**) and B (**4**) merit some discussion. Comparative studies of the cd spectra with alloxanthin (**10**), possessing known chirality (11), were reliable on the absolute configuration at C-3′ of amarouciaxanthin B (**4**). The treatment of amarouciaxanthin B (**4**) with 10% KOH in MeOH gave 6-oxo-isophorone (**6**) and triophaxanthin (**8**). The cd spectrum of (**8**) in Et_2O-2-pentane-EtOH (5:5:2) (EPA) showed weak negative absorptions [nm ($\Delta\epsilon$), 240 (−5.0) and 265 (−2.0)] (Figure 2). Its cd spectrum was similar to that of alloxanthin (**10**) as shown in Figure 2. Therefore, amarouciaxanthin B (**4**) possesses the *R*-configuration at C-3′.

However, the cd spectrum of amarouciaxanthin B (**4**) showed both a strong negative and a fairly strong positive absorption [EPA, nm, ($\Delta\epsilon$), 240 (−35.6) and 278 (+16.8)] (Figure 3). It seems that the configuration at C-6 in amarouciaxanthin B (**4**) contributes large effects to the cd spectrum since the hydroxyl located on C-6 is held between two chromophores, a carbonyl and an oxo-ϵ-end group, as observed in abscisic acid. Therefore, in favorable comparison with the cd spectra of (+)-abscisic acid (**15**) possessing the 6*S*-configuration, amarouciaxanthin B (**4**) was assigned the *S*-configuration at C-6. Furthermore, the additive hypothesis of cd spectra (12) was applied to determine the absolute configuration of amarouciaxanthin B (**4**). That is, (a) to the cd spectrum of *S*-(+)-abscisic acid (**15**) that of (3′*R*)-triophaxanthin (**8**) was added on a graph; (b) those of *R*-(−)-abscisic acid (**16**) and (3′*R*)-triophaxanthin (**8**) were also processed by the same manner. As was expected, the additive cd spectra of the former, (a),

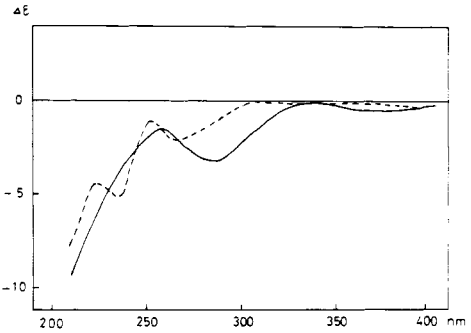


FIGURE 2. Cd spectra of alloxanthin (**10**) (———) and triophaxanthin (**8**) (– – – –) derived from amarouciaxanthin B (**4**) in Et_2O-2-pentane-EtOH (5:5:2) (EPA) at 20°.

was closely similar to that of amarouciaxanthin B (**4**) (Figure 4). It was therefore suggested that the absolute configuration at C-6 in amarouciaxanthin B (**4**) was the same as that of *S*-(+)-abscisic acid (**15**).

Next, the absolute configurations of amarouciaxanthin A (**3**) were confirmed as follows. The cd spectrum of paracentrone (**7**), which was produced by alkaline hydrolysis of **3**, was the same as that of **7** obtained from fucoxanthinol (**14**) in the same manner. The analogous additive cd rule mentioned above for amarouciaxanthin B (**4**) was also applied to determine the configuration at C-6 in amarouciaxanthin A (**3**). Fucoxanthin (**13**), in phytoplankton and sea weed on which the tunicate feeds, seems to be metabolized to fucoxanthinol (**14**), halocynthiaxanthin (**1**), mytiloxanthin (**12**), and eventually to mytiloxanthinone (**2**) (13). At the same time, fucoxanthin (**13**) also seems to be metabolized to amarouciaxanthin B (**4**) via **14** and **3** by a different pathway (Fig-

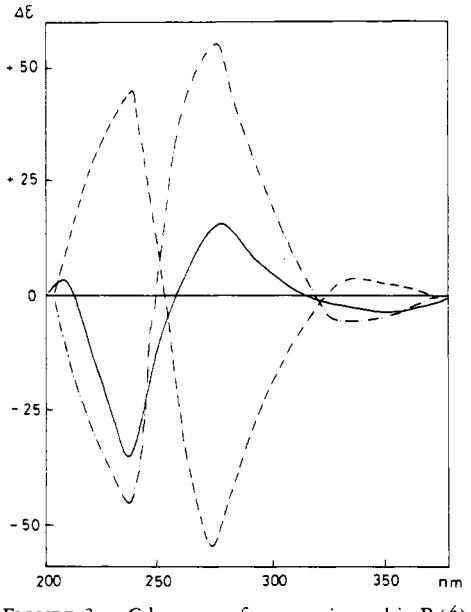


FIGURE 3. Cd spectra of amarouciaxanthin B (**4**) (———), *S*-(+)-abscisic acid (**15**) (– · – · –) and *R*- (−)-abscisic acid (**16**) (– – – –) in Et_2O-2-pentane-EtOH (5:5:2) (EPA) at 20°

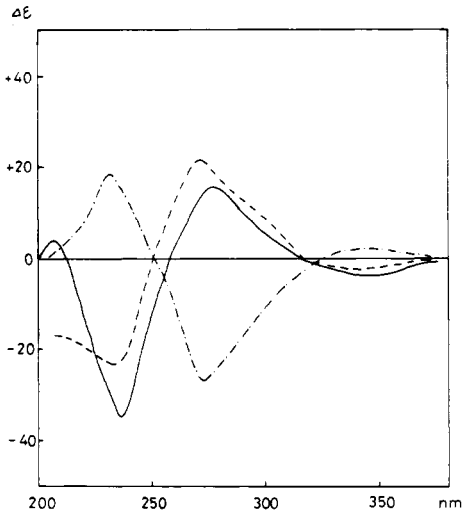


FIGURE 4. Cd spectra of amarouciaxanthin B (**4**) (———), (a) addition of cd spectra of (3*R*)-triophaxanthin (**8**) derived from (**4**) and *S*-(+)-abscisic acid (**15**) (– – – –), and (b) addition of cd spectra of (**8**) derived from (**4**) and *R*-(−)-abscisic acid (**16**) (– · – · –) in Et_2O-2-pentane-EtOH (5:5:2) (EPA) at 20°.

ure 5). Considering these metabolic pathways of fucoxanthin (**13**), these absolute configurations of amarouciaxanthin A (**3**) and B (**4**) are reasonably explained.

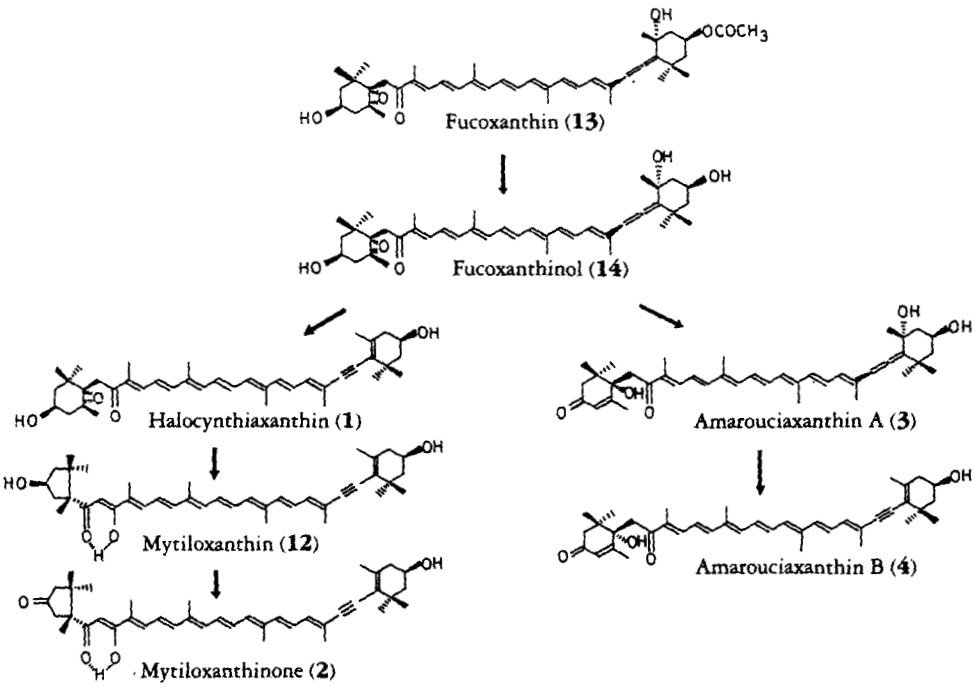


FIGURE 5. Proposed metabolic pathways of fucoxanthin (**13**) in tunicates.

Therefore, amarouciaxanthin A (**3**) was determined to be (6*S*,3′*S*,5′*R*,6′*R*)-6,3′,5′-trihydroxy-4,5,6′,7′-tetradehydro-7,8,5′,6′-tetrahydro-β,β-carotene-3,8-dione and amarouciaxanthin B (**4**) was determined to be (6*S*,3′*R*)-6,3′-dihydroxy-4,5,7′,8′-tetradehydro-7,8-dihydro-β,β-carotene-3,8-dione.

Amarouciaxanthin A (**3**) and B (**4**) weakly inhibited the in vitro cell growth of P-388 leukemia and L-1210 leukemia, and its ED_{50} showed 150 and 300 μg/ml, and 50 and 100 μg/ml, respectively; that of halocynthiaxanthin (**1**) showed 12.5 and 25.0 μg/ml, respectively.

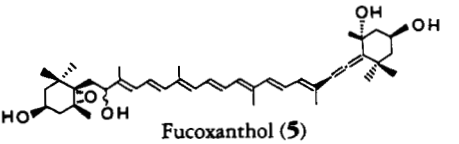
EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were measured on a Yanagimoto micromelting point apparatus and are uncorrected. The uv-visible spectra were recorded in Et_2O with a Shimadzu UV-240 spectrophotometer. The ir spectra were recorded on a Shimadzu IR-27G spectrophotometer in KBr pellet. The ^{1}H-nmr spectra were run at 270 MHz in $CDCl_3$ on a JEOL FX-270 spectrometer with TMS as an internal standard. The ^{13}C-nmr spectrum was taken at 25.1 MHz on the same instrument using pulsed FT in $CDCl_3$ with TMS as an internal standard. The mass spectra were measured with Nippon densi JEOL TMS-01SG, Hitachi M-80, and JEOL JMS-D 300 mass spectrometer.

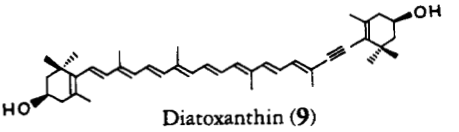
The cd spectra were recorded in EPA [Et_2O-2-pentane-EtOH (5:5:2)] solution at 20° on a Jasco J-500 C spectropolarimeter. Preparative tlc was carried out on a plate (20×20 cm, 0.5 mm thick) coated with silica gel 60 (Merck) using petroleum ether-Me_2CO (7:3) as solvent. For column chromatography, cellulose (Merck) and MgO (Merck) were used separately.

BIOLOGICAL MATERIALS.—*A. pliciferum*, a brilliant red tunicate (6 kg in wet weight), was harvested in July 1982, at the Hamana Lake, Shizuoka Prefecture, Japan; identification was confirmed by Dr. Takashi Tokioka, Emeritus Professor, Seto Marine Laboratories, Kyoto University.

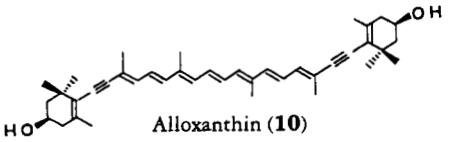
ISOLATION OF CAROTENOIDS.—The Me_2CO extract of the tunicate was concentrated to afford an aqueous solution, which was repeatedly extracted with Et_2O. The upper layer was dried over anhydrous Na_2SO_4 and concentrated to an oil. The oily residue was again dissolved in petroleum ether-ether (1:1).



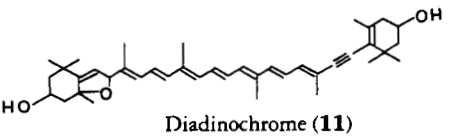
Fucoxanthol (**5**)



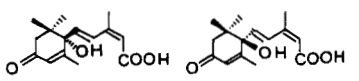
Diatoxanthin (**9**)



Alloxanthin (**10**)



Diadinochrome (**11**)



S-(+)-Abscisic acid (**15**) *R*-(−)-Abscisic acid (**16**)

The extracts were separated into six fractions (bands 1-6) on silica gel with preparative tlc developing with petroleum ether-Me_2CO (7:3) as solvent.

AMAROUCIAXANTHIN B (**4**).—Compound **4** was isolated from band 1 (Rf 0.45∼0.50) (43% of total carotenoids) of the first silica gel preparative tlc. Subsequently, **4** was purified by a cellulose column, eluting with petroleum ether-Et_2O (4:6). Compound **4** was obtained as reddish needles (from petroleum ether-Et_2O), mp 154°; $C_{40}H_{52}O_4$ (M^+, 596); uv-visible λ max Et_2O) 230 nm, 281, and 463; ir ν max (KBr) 3350 (OH), 2165 (-C ≡C-), 1660 and 1650 (conj. C=O), and 960 (*trans*-disubstituted ethylene) cm^{-1}; ms *m/z* (rel. int.) 596 (M^+, 1%), 580 (M^+-16, 0.5), 578 (M^+-18, 1), 564 (M^+-16-16, 0.5), 562 (M^+-16-18, 0.5), 560 (M^+-18-18, 0.5), 504 (M^+-92, 0.5), 486 (M^+-92-18, 0.5), and 444 (M^+-152, base peak, $C_{31}H_{40}O_2$); cd (shown in Figure 3); ^1H nmr ($CDCl_3$) δ 1.05 (3H, s, 16-Me), 1.08 (3H, s, 17-Me), 1.89 (3H, s, 18-Me), 1.95 (3H, s, 19-Me), 1.98 (3H×3, s, 20, 19′ and 20′-Me), 2.35 and 2.48 (2H, ABq, J=18 Hz, 2-CH_2), 2.96 and 3.06 (2H, ABq, J=15 Hz, 7-CH_2), and 5.81 (1H, s, 4-CH=); ^{13}C nmr ($CDCl_3$) ppm 11.6 (q, 19-C), 12.7 (q, 20′-C), 12.9 (q, 20-C), 18.1 (q, 19′-C), 20.7 (q, 18-C), 23.3 (q, 16-C), 24.8 (q, 17-C), 38.7 (t, 7-C), 42.0 (s, 1-C), 49.8 (t, 2-c), 78.5 (s, 6-C), 89.5 (s, 7′-C), 98.6 (s, 8′-C), 120.0 (s, 9′-C), 123.1 (d, 11-C), 125.3 (d, 11′-C), 126.0 (d, 4-C), 129.6 (d, 15′-C), 132.9 (d, 14′-C), 133.2 (d, 15-C), 135.0 (s, 9-C), 135.0 (d, 12′-C), 135.4 (s, 13-C), 137.6 (s, 13′-C), 137.7 (d, 14-C), 138.0 (d, 10′-C), 142.2 (d, 10-C), 147.1 (d, 12-C), 167.9 (s, 5-C), 197.6 (s, 8-C), 203.4 (s, 3-C); *Anal.* calcd. for $C_{40}H_{52}O_4$: C 80.49, H 8.78; Found: C 80.48, H 8.60.

ISOLATION OF DIATOXANTHIN (**9**), ALLOXANTHIN (**10**), DIADINOCHROME (**11**) AND MYTILOXANTHIN (**12**).—Band 2 (Rf 0.35∼0.45) was chromatographed on MgO-Celite 545 (1:1) to give four fractions (14). The first to the third were eluted with Me_2CO [diatoxanthin (**9**)], Me_2CO-MeOH (95:5) [alloxanthin (**10**)], Me_2CO-MeOH (85:15) [diadinochrome (**11**)], respectively, and then the fourth was eluted with MeOH [mytiloxanthin (**12**)].

DIATOXANTHIN (**9**).—Compound **9** was obtained as reddish needles (from petroleum ether) (3% of total carotenoids), visible λ max (Et_2O) 425 nm(sh), 450, and 478; ir ν max (KBr) 3350 (OH), 2165 (-C ≡ C-), and 960 (*trans*-disubstituted ethylene) cm^{-1}; ms *m/z* 566 (M^+, $C_{40}H_{54}O_2$), 548 (M^+-18), 530 (M^+-18-18), and 474 (M^+-92). The physical constants and spectral data of (**9**) coincided with those of an authentic sample (15).

ALLOXANTHIN (**10**).—Compound **10** was obtained as reddish needles (from petroleum ether) (10% of total carotenoids), visible λ max (Et_2O) 428 (sh) nm, 452, and 480; ir ν max (KBr) 3300 (OH), 2165

(-C≡C-), and 960 (*trans*-disubstituted ethylene) cm^{-1}; ms *m/z* 564 (M^+, $C_{40}H_{52}O_2$), 546 (M^+-18), 528 (M^+-18-18), 472 (M^+-92); cd (EPA) nm ($\Delta\epsilon$) 250 (−0.8), 285 (−3.0), 320 (0), 335 (+0.3), and 350 (0). These data were identical with those of alloxanthin obtained from *Halocynthia roretzi* (16).

DIADINOCHROME (**11**).—Compound **11** (2% of total carotenoids) showed λ max (Et_2O) 400 nm, 428 and 454; ms *m/z* 582 (M^+, $C_{40}H_{54}O_3$), 564 (M^+-18) and 502 (M^+-80). The carotenoid was identified on co-tlc with authentic diadinochrome derived from diadinoxanthin obtained from diatoms (9).

MYTILOXANTHIN (**12**).—Compound **12** was isolated as reddish needles (from petroleum ether) (2% of total carotenoids), visible λ max (Et_2O) 469 nm; ir ν max (KBr) 3350 (OH), 1610, 1590, and 1588 (enolic β-diketone) cm^{-1}; ms *m/z* 598 (M^+, $C_{40}H_{54}O_4$), 580 (M^+-18), 562 (M^+-18-18), 506 (M^+-92), 488 (M^+-92-18), 471 (M^+-127), and 401 (M^+-197). These spectral data of **12** were identical to those of mytiloxanthin obtained from *Mytilus edulis* (10).

FUCOXANTHIN (**13**).—Compound **13** (1% of total carotenoids) was isolated from band 3 (Rf 0.30). Vis, ir, ms, ^1H nmr, and cd of (**13**) were identical with those of authentic fucoxanthin (4).

HALOCYNTHIAXANTHIN (**1**).—Compound **1** (1% of total carotenoids) was isolated from band 4 (Rf 0.25). Vis, ir, ms, ^1H nmr, and cd were coincident with an authentic sample from *Halocynthia roretzi* (2).

AMAROUCIAXANTHIN A (**3**).—Compound **3** was obtained from band 5 (Rf 0.20) (7% of total carotenoids) of the first silica gel preparative tlc. Subsequently, **3** was purified by a cellulose column, eluting with petroleum ether-Et_2O (6:4). Compound **3** was crystallized from petroleum ether to give 2 mg as reddish needles, mp 160°; $C_{40}H_{54}O_5$ (M^+, 614); uv-visible λ max (Et_2O) 230 nm, 269, and 455∼460; ir ν max (KBr) 3350 (OH), 1928 (allene), 1660 and 1650 (conj. C=O), and 960 (*trans*-disubstituted ethylene) cm^{-1}; ms *m/z* (rel. int.) 614 (M^+, 1%), 598 (M^+-16, 2), 596 (M^+-18, 2), 582 (M^+-16-16, 4), 578 (M^+-18-18, 4), 560 (M^+-18-18-18, 4), 522 (M^+-92, 2), 462 (M^+-152, base peak, $C_{31}H_{42}O_3$, calcd. 462.3123; Found: 462.3114), 445 (M^+-169, 23) and 444 (M^+-170, 50); cd (EPA) nm ($\Delta\epsilon$) 220 (0), 245 (−44.9), 260 (0), and 270 (+16.7); ^1H nmr ($CDCl_3$) δ 1.81 (3H, s, 19′-Me), 1.95 (3H, s, 19-Me), 1.98 (3H×2, 5, 20 and 20′-Me), and 2.96 and 3.06 (2H, ABq, J=15 Hz, 7-CH_2).

FUCOXANTHINOL (**14**).—Compound **14** (3% of total carotenoids) was isolated from band 6 and showed λ max (Et_2O) 410 (sh) nm, 446, and 470; ir ν max (KBr) 3300 (OH), 1928 (allene), 1660 (conj. C=O), and 960 (*trans*-disubstituted ethylene) cm^{-1}; ms *m/z* 616 (M^+, $C_{40}H_{56}O_5$). **14** was identical with authentic fucoxanthinol obtained from algae *Sargassum fulvellum* (4) on co-tlc.

ALKALINE TREATMENT OF AMAROUCIAXANTHIN A (**3**) AND B (**4**).—A suspension of **3** (2 mg) in 10% KOH/MeOH (10 ml) was stirred for 1 h at 37°. The resulting solution was extracted with Et_2O (20 ml×2). The extract solution was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated in vacuo in N_2 below 40° in the dark. The residue afforded two bands on silica gel preparative tlc (Merck), developing with petroleum ether-Me_2CO (7:3). Each band, extracted with petroleum ether-Me_2CO (1:1), evaporated in vacuo in N_2 to give 6-oxo-isophorone (**6**) (from upper band) and paracentrone (**7**) (from lower band).

In a similar procedure, **6** and triophaxanthin (**8**) were afforded from (**4**).

6-OXO-ISOPHORONE (**6**).—Compound **6** showed uv λ max (Et_2O) 238 nm; ms *m/z* 152 (M^+, $C_9H_{12}O_2$). Compound **6** was completely identical with an authentic synthetic sample of (**6**) by co-glc (20% PEG20M/Chromosorb W, 250°).

PARACENTRONE (**7**).—Compound **7** showed uv λ max (Et_2O) 425(sh), 444, and 468 nm; ms *m/z* 462 (M^+, $C_{31}H_{42}O_3$), 444 (M^+-18), 426 (M^+-18-18), and 411 (M^+-18-18-15); ^1H nmr ($CDCl_3$) δ 1.08 (3H, s), 1.34 (6H, s), 1.81 (3H, s), 1.94 (3H, s), 1.98 (6H, s), and 2.35 (3H, s).

TRIOPHAXANTHIN (**8**).—Compound **8** showed uv λ max (Et_2O) 447 and 470(sh) nm; ms *m/z* 444.2983 (M^+, calcd. for $C_{31}H_{40}O_2$: 444.3018); ^1H nmr ($CDCl_3$) δ 1.14 (3H, s), 1.20 (3H, s), 1.93 (6H, s), 1.99 (9H, s), and 2.36 (3H, s); cd (EPA) nm ($\Delta\epsilon$) 240 (−5.2), 250 (−2.0), and 280 (−3.0).

CYTOTOXIC ACTIVITY AGAINST P-388 AND L-1210.—P-388 and L-1210 leukemia cells were cultured in the presence of various concentrations of amarouciaxanthin A (**3**) or B (**4**) or halocynthiaxanthin (**1**) in Eagle's essential medium supplemented with 10% calf serum, 5 μg/ml cefazolin, and 100 μg/ml streptomycin at an initial cell density of 6×10^2 cells/ml at 37° in an incubator with 7% CO_2; after 4 days cultivastion, the cell density was observed under a microscope and the ED_{50} values were determined.

ACKNOWLEDGMENTS

The authors wish to acknowledge Mr. T. Maoko, Kyoto Pharmaceutical University, for his advice

and encouragement in the performance of this work, The technical assistance of Mrs. M. Shimizu, Y. Kanaoka, M. Takino, and I. Shimizu is acknowledged.

LITERATURE CITED

1. K. Nishibori, *Publ. Seto Mar. Biol. Lab.*, **7**, 181 (1958).
2. T. Matsuno and M. Ookubo, *Tetrahedron Lett.*, 4659 (1981).
3. T. Matsuno and M. Ookubo, *Chem. Lett.*, 1605 (1982).
4. R. Bonnett, A.K. Mallams, A.A. Spark, J.L. Tee, B.C.L. Weedon, and A. McCormick, *J. Chem. Soc. (C)*, 429 (1969).
5. G. Galasko, J. Hora, T.P. Toube, B.C.L. Weedon, D. André, M. Barbier, E. Lederer, and V.R. Villanueva, *J. Chem. Soc. (C)*, 1264 (1969).
6. J.W. McBeth, *Comp. Biochem. Physiol.*, **41B**, 55 (1972).
7. D.J. Chapman, *Phytochemistry*, **5**, 1331 (1966).
8. A.K. Mallams, E.S. Waight, B.C.L. Weedon, D.J. Chapman, F.T. Haxo, T.W. Goodwin, and D.M. Thomas, *Chem. Comm.*, 301 (1967).
9. F.P. Healey, J. Coombs, and B.E. Volcani, *Archiv für Mikrobiologie*, **59**, 131 (1967).
10. A. Khare, G.P. Moss, and B.C.L. Weedon, *Tetrahedron Lett.*, 3921 (1973).
11. R. Berger, G. Borch, and S. Liaaen-Jensen, *Acta Chem. Scand.*, **B31**, 243 (1977).
12. S. Liaaen-Jensen, in: *Fortschritte der Chemie organischer Naturstoffe*, vol. 39. Ed. by W. Herz, H. Grisebach, and G.W. Kirby, New York, Springer-Verlag, 1980, p. 123.
13. M. Ookubo and T. Matsuno, *Comp. Biochem. Physiol.*, **81B**, 137 (1985).
14. T. Matsuno, M. Ookubo, T. Nishizawa, and I. Shimizu, *Chem. Pharm. Bull.*, **32**, 4309 (1984).
15. T. Matsuno and T. Maoka, *Bull. Japan. Soc. Sci. Fish.*, **47**, 377 (1981).
16. Y. Tsuchiya and Y. Suzuki, *Tohoku J. Agric. Res.*, **10**, 367 (1960).

Received 13 February 1985