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ON THE ABSOLUTE CONFIGURATION OF 19′-HEXANOYLOXYFUCOXANTHIN*

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Key Word Index—*Coccolithus huxleyi*; Haptophyceae; stereochemistry (3*S*, 5*R*, 6*S*, 3′*S*, 5′*R*, 6′*S*)-19′-*n*-hexanoyloxyfucoxanthin; minor carotenoids diadinoxanthin and 3′-desacetyl-19′-*n*-hexanoyloxyfucoxanthin (first isolation).

Abstract—The esterifying C_6-acid in 19′-hexanoyloxyfucoxanthin has been identified as *n*-hexanoic acid by GLC of the methyl ester. Ozonolysis of 19′-*n*-hexanoyloxyfucoxanthin 3-benzoate provided the *n*-hexanoyloxy derivative of the allenic ketone produced from fucoxanthin 3-benzoate. NMR and CD correlation of the ozonolysis products and NMR of the native carotenoids provided the basis for assignment of the same absolute configuration of the 19′-*n*-hexanoyloxy derivative (3*S*, 5*R*, 6*S*, 3′*S*, 5′*R*, 6′*S*) as for fucoxanthin. Biosynthetic implications are considered. CD data for 19′-*n*-hexanoyloxyfucoxanthin, fucoxanthin and some derivatives thereof are reported. Previously unreported minor carotenoids in *Coccolithus huxleyi* were diadinoxanthin and 3′-desacetyl 19′-*n*-hexanoyloxyfucoxanthin.

INTRODUCTION

Recently 19′-hexanoyloxyfucoxanthin (**1**) was shown to be the major carotenoid of the haptophyte alga *Coccolithus huxleyi* (clone BT-6, Guillard) [1].

We now report conclusive identification of the esterifying C_6-acid and studies on the absolute configuration of this new fucoxanthin (**2**) derivative.

RESULTS AND DISCUSSION

The carotenoid composition of the present batch of *Coccolithus huxleyi* differed from earlier findings [1, 2] in the respect that the apocarotenoid 19-hexanoylparacentrone 3-acetate and diatoxanthin were not detected, whereas diadinoxanthin (**3**) [3, 4] was a minor carotenoid (7% of the total carotenoid). Diadinoxanthin (**3**) was identified by co-chromatography with an authentic sample, electronic and mass spectra of the free diol (**3**) and the diacetate **3a**. Diadinoxanthin (**3**) could only be quantitatively separated from the major carotenoid 19′-hexanoyloxyfucoxanthin (**1**) after acetylation. Isolated for the first time was a minor carotenoid (2% of total carotenoids) with adsorptive, electronic and mass spectral properties consistent with a 3′-desacetyl-19′-hexanoyloxyfucoxanthin (**4**).

19′-Hexanoyloxyfucoxanthin (**1**) was submitted to alkaline hydrolysis. The esterifying acids were analyzed by GLC and the C_6-acid conclusively identified as *n*-hexanoic acid before and after methylation.

Concerning the absolute configuration of **1**, comparative studies with fucoxanthin (**2**) of known chirality [5] were resorted to. *A priori* CD spectra of fucoxanthin (**2**) and fucoxanthin acetate (**2a**) and particularly of fucoxanthol (**5**) and fucoxanthol triacetate (**5a**) were expected to be dictated mainly by the geometry of the allenic end group. If the influence of the achiral 19′-substituent in 19′-hexanoyloxyfucoxanthin (**1**) and its derivatives was negligable, CD comparison of derivatives from the two series should be meaningful. For this purpose 19′-hexanoyloxyfucoxanthin 3-acetate (**1b**), 19′-hydroxyfucoxanthol (**6**) and 19′-hydroxyfucoxanthol tetraacetate (**6a**) and the fucoxanthin derivatives mentioned above were prepared. CD data are given in Fig. 1. ORD and CD data for fucoxanthin (**2**) have been reported previously [6, 7a, 7b]. In spite of six chiral centra the Cotton effect of these carotenoids is very weak and sensitive to impurities. No definite conclusions may be drawn but

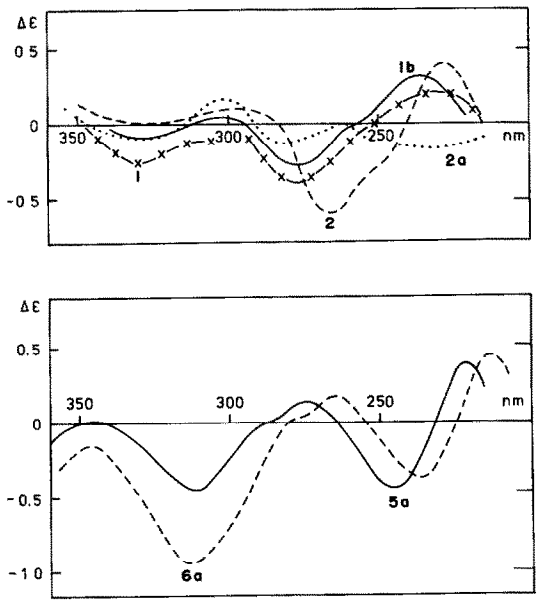


Fig. 1. CD spectra in EPA (Et_2O, isopentane, EtOH 5:5:2) solution of fucoxanthin (**2**), fucoxanthin 3-acetate (**2a**), 19′-hexanoyloxyfucoxanthin (**1**) and 19′-hexanoyloxyfucoxanthin 3-acetate (**1b**), upper part. CD spectra in EPA solution of fucoxanthol triacetate (**5a**) and 19′-hydroxyfucoxanthol tetraacetate (**6a**), lower part.

* Part 20 in the series 'Algal Carotenoids', Part 19, Buchecker, R. and Liaaen-Jensen, S. *Phytochemistry* in press.

Table 1. NMR signals (δ, $CDCl_3$) associated with the end groups of fucoxanthin (2), 19′-*n*-hexanoyloxyfucoxanthin (1) and the allenic ketones 8 and 7

Compound	End group A			End group B		
	Me-16, 17	Me-18	CH_2-7	Me-16′-17′	Me-18′	CH-8′
Fucoxanthin (2)	0.97	1.20	2.58 *d*	1.07	1.32	6.06
	1.04		3.68 *d*	1.23		
19′-*n*-Hexanoyloxyfucoxanthin (1)	0.97	1.21	2.64 *d*	1.10	1.37	6.14
	1.04		3.72 *d*	1.26		
Ketone 8 (from 2)				1.15	1.42	5.90
				1.42		
Ketone 7 (from 1)				1.18	1.45	5.95
				1.42		

approximate agreement in the CD spectra of 19′-hexanoyloxyfucoxanthin (1) and the corresponding 3-acetate (1b) (Fig. 1) suggests that acetylation of ring A does not alter the CD. Inconsistency between the CD of fucoxanthin (2) and the 19′-hexanoyloxyfucoxanthin derivatives 1 and 1b of the same chromophore could be due to different chirality or the effect of the 19′-hexanoyloxy substituent on the detailed geometry of end group B; cf. the small difference in electronic spectra. Rough agreement above 370 nm in the Cotton effect of the $LiAlH_4$ reduced derivatives fucoxanthol triacetate (5a) and 19′-hydroxyfucoxanthol tetraacetate (6a) with a smaller 19′-substituent may favour similar geometry of end group B.

For conclusive assignments the native 19′-hexanoyloxyfucoxanthin (1), converted to the 3-benzoate (1c), was submitted to ozonolysis. Ozonolysis provided the allenic ketone 7, containing the acetoxy and *n*-hexanoyloxy functions, thus confirming the previous allocation of the 19′-hexanoyloxy substituent in 1. The ketone 7, characterized by UV, IR, NMR, MS and CD spectra, corresponds to the allenic ketone 8 previously obtained by ozonolysis of fucoxanthin (2) [8, 9] and for which the stereochemistry has been defined from X-ray diffraction data [10]. The allenic ketone 8 was also prepared here in parallel experiments with fucoxanthin 3-benzoate (2b).

Close agreement in chemical shifts of the NMR spectrum for methyl signals associated with the allenic end groups in fucoxanthin (2), 19′-hexanoyloxyfucoxanthin (1) and the allenic ketones 7 and 8 (Table 1) and CD data for ketones 7 and 8 with the same chromophores (Fig. 2) confirm that the three chiral centres of the allenic end group have the same absolute configuration.

Ozonolysis products originating from end group A of the fucoxanthin (2) molecule have not been reported [8, 9]. In the present work the same, unidentified, spectroscopically characterized products) 9 obtained by ozonolysis of fucoxanthin 3-benzoate (2b) and 19′-hexanoyloxyfuxanthin 3-benzoate (1c) is likely to originate from the benzoylated end group. However, no stereochemical information could be obtained from this optically inactive product.

Trans relative configuration between the hydroxy and epoxy substituents of ring A in 19′-hexanoyloxyfucoxanthin is concluded from the exact agreement in chemical shifts of the 16, 17 and 18-methyl groups of fucoxanthin (2) and 19′-hexanoyloxyfucoxanthin (1), and correspondance for the 7-methylene groups. Attempted conversions of 19′-hydroxyfucoxanthol (6) to the C-8 epimeric 19′-hydroxy neochromes (10a) with acid [5] for CD-correlation was only partly successful, the reaction proceeding in low yield, possibly accompanied by dehydration of the 5′-hydroxy function.

In conclusion the nature of the esterifying *n*-hexanoic acid and the 3′*S*, 5′*R*, 6′*S* absolute configuration of 19′-hexanoyloxyfucoxanthin (1) is unequivocally established in the present work. The relative *trans* configuration of the 3-hydroxy/5,6-epoxy function is based on NMR considerations, whereas preference for 3*S*, 5*R*, 6*S* configuration (1a) rests on analogy with fucoxanthin (2) and all other carotenoids containing ring B [7, 11], including diadinoxanthin (3) [4] isolated as a minor carotenoid from *Coccolithus huxleyi*.

For chemotaxonomic considerations the same absolute configuration of the six chiral centres of 1a and 2 points towards a common biosynthetic route for fucoxanthin (2) and 19′-hexanoyloxyfucoxanthin (1a). The biosynthesis of the latter requires presumably two extra steps (allylic hydroxylation and *n*-hexanoylation) from fucoxanthin (2). So far the presence of fucoxanthin (2) or 19′-hydroxyfucoxanthin has not been documented in *Coccolithus huxleyi*, which exhibits in algal context a remarkable dominance (around 90%) of this structurally complex carotenoid (1a).

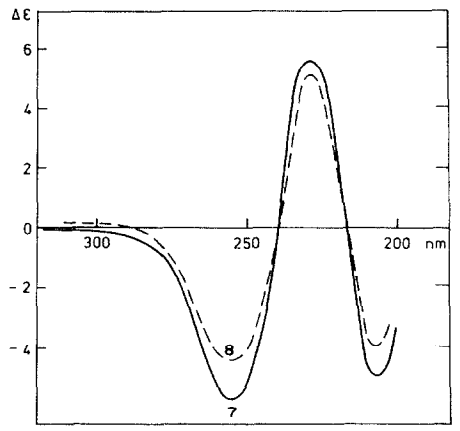
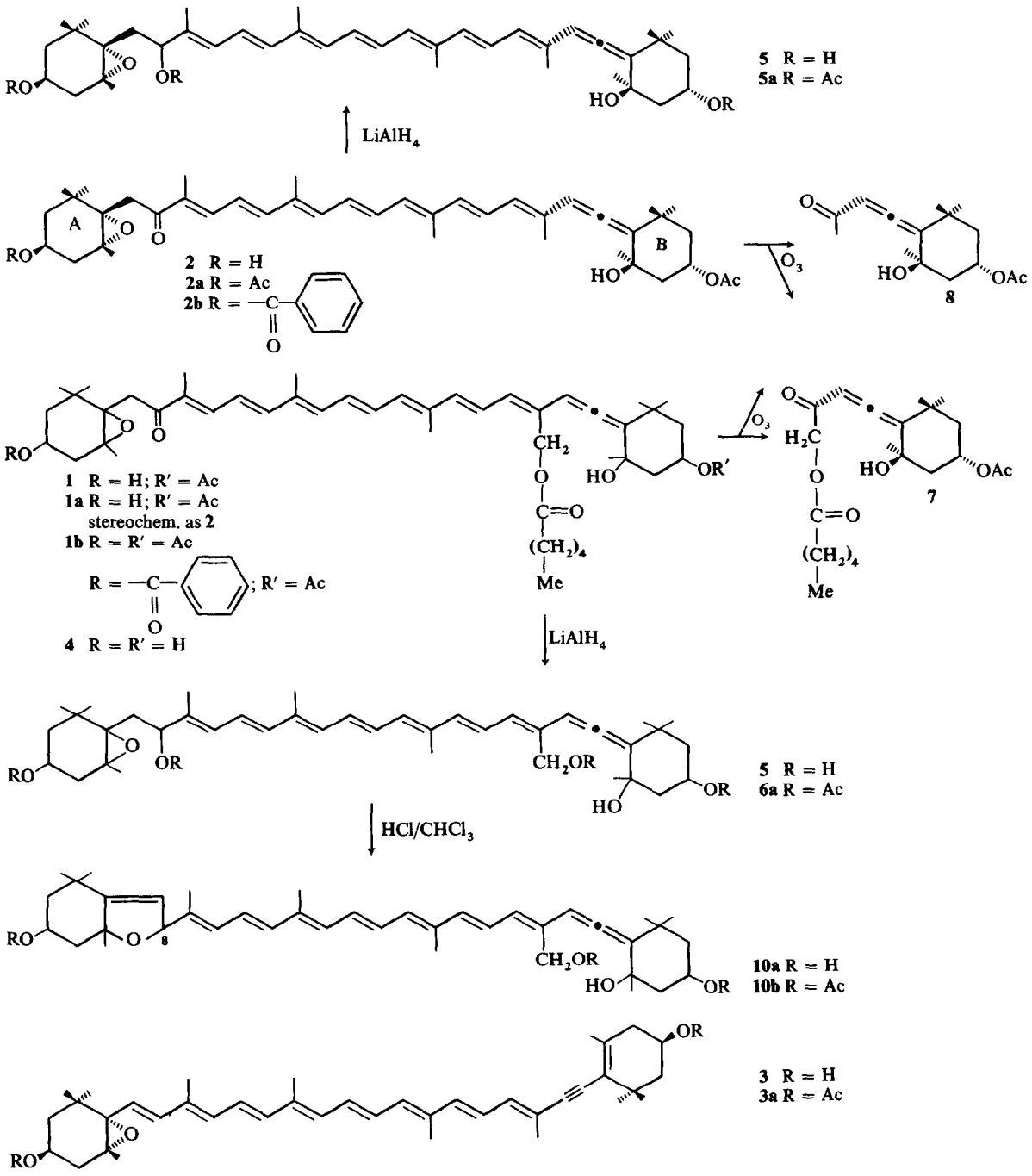


Fig. 2. CD spectra in EPA solution of the allenic ketones 8 and 7, obtained by ozonolysis of fucoxanthin 3-benzoate (2b) and 19′-hexanoyloxy fucoxanthin 3-benzoate (1c).



EXPERIMENTAL

Biological material. Coccolithus hyxleyi clone BT-6, Guillard, was grown in large scale culture [14]; yield 65 g lyophilized cells.

Pigment isolation. Pigments were extracted with Me_2CO–MeOH (1:1), transferred to Et_2O and chromatographed repeatedly on TLC (Si gel G: Me_2CO–hexane (35:65) and Si gel columns to effect separation from chlorophyll derivatives; total yield of crude carotenoids 222 mg (0.34% of dry wt). Estimated carotenoid composition was β,ε-carotene and β,β-carotene 2% of total carotenoid, diadinoxanthin (**3**, 7%), 19′-hexanoyloxyfucoxanthin (**1**, 89%) and 3-desacetyl-19′-hexanoyloxyfucoxanthin (**4**, 2%). Complete separation of diadinoxanthin (**3**) and 19′-hexanoyloxyfucoxanthin (**1**) was only achieved after acetylation.

Diadinoxanthin (**3**). $\lambda_{max}^{Et_2O}$ 420, 443 and 473 nm; *m/e* 582 (M) and fragmentation pattern as obtained for authentic **3** [3], R_f = 0.72 (Schleicher & Schüll no. 287 = S & S 287: Me_2CO–hexane (2:8), inseparable from authentic **3**. Diadinoxanthin diacetate (**3a**) had *m/e* 666 (M) and predicted fragmentation pattern [3, 15].

3′-Desacetyl-19′-hexanoyloxyfucoxanthin (**4**). λ_{max} as **1**, but more strongly adsorbed. Purified by TLC (kieselgel G; Me_2CO–hexane (1:1) and kieselgel columns (eluent Me_2CO–hexane (2:3) **4** had $\lambda_{max}^{Et_2O}$ (420), 444 and 472 nm; MS *m/e*: 730 (M), M-18, M-18-16, M-18-18, M-18-18-18, M-18-92, M-16-114, M-18-114, M-18-16-114, M-18-18-114, M-18-18-116.

19′-*Hexanoyloxyfucoxanthin* (**1**). R_f, λ_{max}, MS and NMR as previously reported [1] and Table 1; CD Fig. 1. Alkaline hydrolysis of **1** (12.5 mg) in 5% KOH in Et_2O–MeOH (1:1) for 5 hr gave during extractive isolation from the acidified hypophase *n*-hexanoic acid, converted to the methyl ester with 5% HCl/MeOH. The identity was proved by GLC on a 5% TCEP column using methyl propionate, methyl *n*-butyrate, methyl isovalerate, methyl *n*-valerate and methyl *n*-hexanoate as references, isothermal at 49°. Furthermore, the free C_6-acid co-chromatographed with *n*-hexanoic acid on a 3% OV-225 column at 70°.

19′-*Hexanoyloxyfucoxanthin* 3-*acetate* (**1b**). Prepared by standard acetylation of **1** had properties as previously reported [1]; $\lambda_{max}^{Me_2CO}$ (420) 444 and 470 nm; % III/II [16] = 26, cf. 0% for **2**; CD (EPA) Fig. 1.

19′-*Hexanoyloxyfucoxanthin* 3-*benzoate* (**1c**). **1** (125 mg) was benzoylated in the same manner as fucoxanthin (**2**); yield 110 mg **1c**. **1c** (110 mg) in EtOAc (100 ml) was ozonized at −60° for 10 min until decolourized. H_2O was added and the pH adjusted with NH_3 to pH 7. Products were extracted with Et_2O, the hypophase acidified and re-extracted with Et_2O. Combined, washed Et_2O extracts were chromatographed (TLC, Si gel: Me_2CO–hexane (1:1) providing two major products **9** (R_f = 0.78) and **7** (R_f = 0.30), rechromatographed for characterization. Product **9** had λ_{max}^{hexane} 225 and 275 nm; *m/e* 390.2769 ($C_{24}H_{38}O_4$ = M?), 279.1598 ($C_{16}H_{23}O_4$); NMR ($CDCl_3$): δ 0.93 *s*, 1.08 *s*, 1.36 *s*, 1.41 *s*, 4.25 *d*, 7.4–7.8 (aromatic H); CD (EPA) inactive. Product **7** had $\lambda_{max}^{Et_2O}$ 205, 230 nm, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 2960, 2940, 2870 (CH), 1940 (allene), 1735 (ester), 1700 (C=O), 1600, 1460, 1420, 1380, 1250 (ester), 1150 (tert. OH), 1100, 1080, 1035 (ester), 950, 910, 860, 820, 760 and 720; NMR ($CDCl_3$): δ 0.90 *ca* 3H, *t* Me–CH_2), 1.18 (3H, *s*, one *gem.* Me), 1.41 (3H, *s*, one *gem.* Me), 1.45 (3H, *s*, Me—C—O), 2.02 (3H, *s*, acetate), 2.30 (2H, *t*, O—CO—C<u>H</u>$_2$—CH_2—), 4.81 (2H, *s*, CH_2—O) and 5.95 (1H, *s*, allene); MS *m/e*: 380.21988 (M = $C_{21}H_{32}O_6$), M-15, M-16, 320.1982 (M—MeCOOH), 264.1359 (M—$Me(CH_2)_4$-COOH); CD Fig. 2

19′-*Hydroxyfucoxanthol* (**6**). **1** (9 mg) reduced with $LiAlH_4$ gave **6** in quantitative yield with properties as previously reported [1]. **1** (4.0 mg) in ethanol-free $CHCl_3$ (5 ml) was treated with 0.05 N HCl in $CHCl_3$ (5 drops) for 10 min; pigment recovery 48% after transfer to Et_2O. Chromatography on acetylated polyamide gave several minor and one major, yellow product (21% of recovered carotenoid), further purified by TLC (Si gel. Me_2CO–hexane (1:1), $\lambda_{max}^{Et_2O}$ 398, 420 and 480 nm, R_f = 0.73 (S & S 287; Me_2CO–hexane (3:7) relative to 0.54 for the pentol **6**. After acetylation a chromatographically homogeneous product (TLC. Si gel: Me_2CO–hexane (1:1) with unchanged λ_{max}; MS *m/e*: 724 (M?), 644 (M-60), 263 (homopyrylium), 223 (furylium); expected for 19-acetoxy neochrome diacetate (**10b**) M = 742; insufficient for further experiments.

19′-*Hydroxyfucoxanthol* 3,8,3′,19′-*tetraacetate* (**6a**). Was prepared by acetylation of **1**, had properties as previously reported [1]; CD Fig. 1.

Reference compounds. Fucoxanthin (**2**), *ex Fucus vesiculosus*; CD (Fig. 1) was in good agreement with reported data (**7b**). *Fucoxanthin acetate* (**2a**), prepared by standard acetylation of **2**; *Fucoxanthin benzoate* (**2b**) prepared from **2** (310 mg) by published procedure [8] and purified by TLC (Si gel: Me_2CO–hexane (1:4) had R_f = 0.71 (S & S 287; Me_2CO–hexane (1:9), λ_{max} as **2**. **2b** (87 g) was ozonized in the same manner as **1c**. Two major products were isolated by TLC (Si gel). Product **9** (R_f = 0.59. Me_2CO–hexane (1:4), $\lambda_{max}^{Et_2O}$ 225, 270 nm; IR ν^{KBr} cm^{-1} 3090 (aromatic CH), 2980, 2960, 2930 (CH), 1735 (ester), 1600, 1590, 1460, 1380, 1270 (ester), 1130, 1070, 1040, 960, 750 and 710; NMR ($CDCl_3$) as given for **9** under **1c**; MS *m/e* 390.2771 ($C_{24}H_{38}O_4$ = M?), 279.1595 ($C_{16}H_{23}O_4$); CD inactive. Product **8** had R_f = 0.26, Me_2CO–hexane (2:3); $\lambda_{max}^{Et_2O}$ 225 nm; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 3000, 2960, 2920 (CH), 1939 (allene), 1720 (acetate), 1675 (C=O), 1600, 1580, 1450, 1365, 1320, 1270, 1240, 1175, 1120 (tert. OH), 1075, 1025, 970, 960, 920, 860, 820, 760 and 720; NMR ($CDCl_3$): δ 1.15 (3H, *s*, one *gem.* Me), 1.42 (6H, *s*, one *gem.* Me, Me—C—O), 2.02 (3H, *s*, acetate), 2.17 (3H, *s*, Me—C=O), 5.90 (1H, *s*, allene); MS *m/e*: 266.1523 ($C_{15}H_{22}O_4$ = M), 206.1302 (M—MeCOOH). *Fucoxanthol* (**5**). **5** was prepared from **2** (4 mg) by $LiAlH_4$-reduction, followed by TLC purification (Si gel: Me_2CO–hexane (1:1). *Fucoxanthol triacetate* (**5a**) was prepared by standard acetylation of **5**, purified by TLC (Si gel: Me_2CO–hexane (1:4): λ_{max}(Me_2CO) 410, 424, 450 nm % III/II [16] = 95%; CD (EPA) Fig. 1

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