Phytochemistry, 1976, Vol. 15, pp. 529–532. Pergamon Press. Printed in England.

NEW FUCOXANTHIN-RELATED CAROTENOIDS FROM *COCCOLITHUS HUXLEYI**

N. ARPIN†, W. A. SVEC‡ and S. LIAAEN-JENSEN

Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway

(*Received* 11 *June* 1975)

Key Word Index—*Coccolithus huxleyi*; Haptophyceae; new carotenoids; 19′-hexanoyloxyfucoxanthin; 19-hexanoyloxyparacentrone.

Abstract—The main carotenoid of *Coccolithus huxleyi* is shown to be 19′-hexanoyloxyfucoxanthin by spectroscopic (electronic IR, ^{1}H NMR, mass) evidence and chemical reactions. A minor carotenoid had properties compatible with the apo-carotenoid 19-hexanoyloxyparacentrone.

INTRODUCTION

The carotenoid composition of the haptophyte alga *Coccolithus huxleyi* (clone BT-6, Guillard) was recently found [1] to consist of α-carotene (1%), β-carotene (2%), the acetylenic diatoxanthin (10%) and a major unknown carotenoid (87%), different from fucoxanthin (**1**).

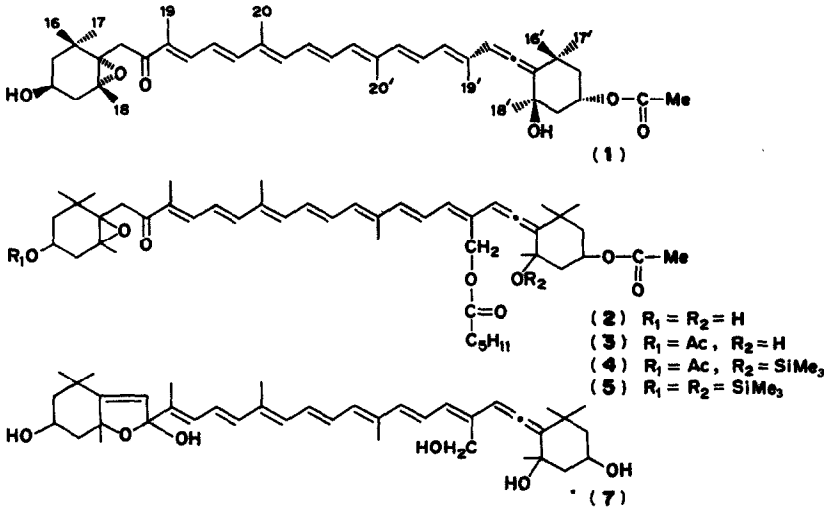
We now report the structural elucidation of the unidentified carotenoid using pigment isolated in the previous study [1] and further material isolated from *C. huxleyi* (strain BT-6). From the latter culture was also isolated a new apo-carotenoid.

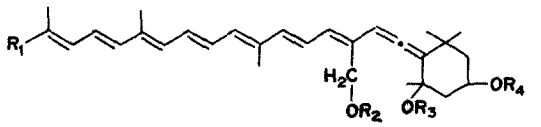
* No. XVI in the series Algal Carotenoids. No. XV *Acta Chem Scand.* (in press). † On leave of absence from Departement de Biologie Végetale, Service de Phytochimie et Phytophysiologie, Universitè-de Lyon, 69-Villeurbanne, France. ‡ On leave of absence from Chemistry Division, Argonne National Laboratory, Argonne, IL 60439, U.S.A.

RESULTS AND DISCUSSION

The major carotenoid (**2**) exhibited electronic spectra identical with those of fucoxanthin (**1**) ex *Fucus vesiculosus*. The ^{1}H NMR spectrum showed methyl signals (see Table and Experimental) consistent with the end groups of fucoxanthin (**1**) [2], and the chemical shifts of the acetate methyl and of the in-chain methyl groups also corresponded to those of (**1**), except that the singlet of the 19′-methyl group (for numbering see **1**, Scheme 1) was replaced by a two proton singlet at δ 4·78, compatible with an esterified primary hydroxy group. Furthermore, the methylene at C-7 occurred as a double doublet as for fucoxanthin [2]. The IR spectrum of **2** further demonstrated the presence of allene, conjugated carbonyl and ester function(s).

The carotenoid (**2**) on standard acetylation provided a monoacetate (**3**, actually a diacetate since (**2**) itself represents a natural acetate) demonstrating the presence of a primary or secondary hydroxy group. This acetate (**3**)





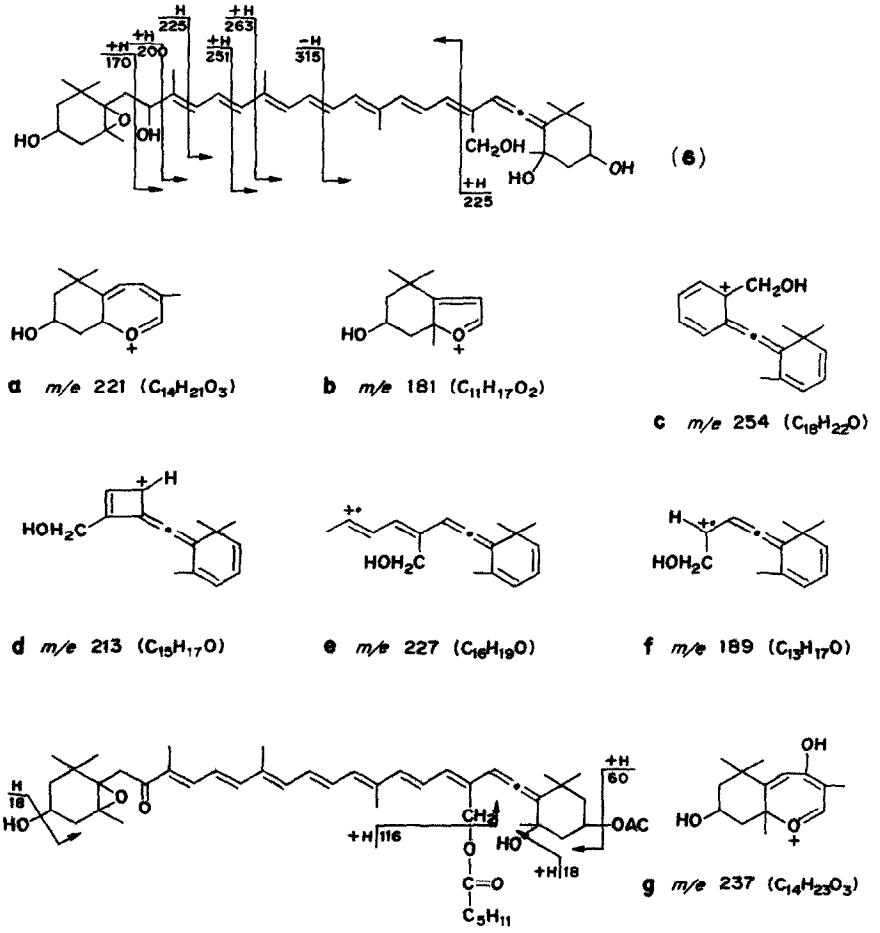
(8) R_1 = MeCO, R_2 = $C_5H_{11}CO$, R_3 = H, R_4 = Ac
(9) R_1 = MeCO, R_2 = $C_5H_{11}CO$, R_3 = $SiMe_3$, R_4 = Ac
(10) R_1 = MeCHOH, R_2 = R_3 = R_4 = H
(11) R_1 = MeCO, R_2 = R_3 = R_4 = H

on silylation gave slowly a mono (trimethylsilyl) ether (4), confirming the presence of a sterically hindered tertiary hydroxy group in (2). In agreement with these results the carotenoid (2) gave a di(trimethylsilyl) ether (5) on silylation.

$LiAlH_4$-reduction of (2) gave the reduction product 6 (Scheme 1) with aliphatic octaene chromophore. The pentol character of this reduced compound followed from its polarity and acetylation to the tetraacetate, which on silylation provided the tetraacetate mono (trimethylsilyl) ether. Under the reaction conditions used the epoxyde was not opened [2,3].

Molecular and fragment ions of the above derivatives on electron impact were consistent with the above conclusion as to the number and type of functional groups present. The mass spectrum of the pentol, rationalized in Scheme 1, further confirms the 19'-hydroxyfucoxanthol assignment. The rationalizations are largely based on exact mass measurements. As expected several combination losses with water are observed. Loss of 92, but not 106, mass units [4] supports assignment of the chromophore. Homopyryllium **a** and furyllium **b** ions [4] and several in-chain cleavages confirm the epoxidic end group. Ions derived from the allenic end group were less abundant. However, fragment ions of elementary composition compatible with the monooxy species **c**, **d**, **e** and **f** may be associated with the allenic end groups, by analogy with fragment ions previously rationalized for peridinin [5].

The natural carotenoid (2) had a weak molecular ion at *m/e* 772 compatible with $C_{48}H_{68}O_8$, supported by exact mass measurements of M-18 (H_2O) = $C_{48}H_{66}O_7$. Diagnostically useful fragment ions occurred at *m/e* 694 = $C_{46}H_{62}O_5$ (M-H_2O-HAc), *m/e* 662 = (M-H_2O-C_6H_5Me)? *m/e* 658 = $C_{42}H_{58}O_6$ = M-$C_6H_{10}O_2$, *m/e* 656 = $C_{42}H_{56}O_6$ = M-$C_6H_{12}O_2$, *m/e* 638 = $C_{42}H_{54}O_5$ = M-H_2O-$C_6H_{12}O_2$, *m/e* 237 = $C_{14}H_{21}O_3$ (*g*) and *m/e* 221 (**a**?). The loss of $C_6H_{10}O_2$ is attributed to the elimination of hexanoic acid from the C-19' ester function with hydrogen transfer from the charged fragment, and the loss of $C_6H_{12}O_2$ to similar cleavage but with hydrogen transfer in the opposite di-



Scheme 1. Molecular and fragment ions in the MS of the pentol 6.

rection. Evidence for the same cleavage was found in the mass spectra of the acetate (3) and the di(trimethylsilyl) ether (4). The methylene group α to the carbonyl in this ester function is recognized as a presumed triplet at δ 8·2 in the ^{1}H NMR spectrum of (2). Regarding the further structure of the C_6-carboxylic acid involved the signals in the methyl region of (2) favour unbranched acid.

19-Hexanoyloxyfucoxanthin (2), like fucoxanthin (1), produced a strong blue colour with conc. HCl in ether, and was converted to products of shorter chromophore on weak alkali treatment [6]. Preliminary studies demonstrated conversion to an instable product with octaene chromophore and properties compatible with the cyclic hemiketal (1).

This is the first finding of an allenic carotenoid with oxygenated methyl group adjacent to the allene. A 19′-hydroxy-methyl group has previously been claimed for the allenic vaucheriaxanthin [7], but this has subsequently been disproved [8]. Slow silylation of the C-5′ hydroxy group of the C-19′ esters is consistent with the C-19′ substitution. Noteworthy is also enhancement of the allenic stretching vibration at 1930 cm^{-1} of (2) relative to fucoxanthin (1) with no C-19′ substituent. Exceptionally strong absorption of (2) at 1390 cm^{-1} must also be connected with this new structural element. Besides peridinin and derivatives [9,10] the main carotenoid of *C. huxleyi* represents the most complex carotenoid structure encountered. Its stereochemistry is being subjected to further studies [11].

A minor carotenoid (8), with the same chromophore, but less strongly adsorbed than (2), had molecular ion at *m/e* 618, compatible with $C_{39}H_{54}O_6$ and fragment ions consistent with losses of water, acetic acid and hexanoic acid. This carotenoid gave no acetate under standard acetylation conditions, but provided a mono (trimethylsilyl) ether (9) on silylation. $LiAlH_4$-reduction provided (10) with octaene chromophore. Saponification of (8) with 0·1% KOH gave a more polar product (11) with unchanged chromophore.

These data are consistent with the methyl ketone formulation 19′-hexanoyloxyparacentrone (8). The biosynthetic formation of (8) from 19′-hexanoyloxyfucoxanthin (10) may be rationalized in the same way as the biosynthesis of paracentrone [12] from fucoxanthin (1) [13].

EXPERIMENTAL

Materials and methods were those commonly employed in this laboratory [14,15]. When elementary composition of fragment ions are given these were secured by accurate mass measurements.

Biological sources. Extracts left from a previous cultivation of *Coccolithus huxleyi*, clone BT-6, Guillard [1] (Batch 1) and an Me_2CO–MeOH (1:1) extract freshly prepared from *C. huxleyi*, grown in large-scale culture [16], (Batch 2, 83 g lyophilized cells) was used. (2) was the principle carotenoid in both batches. The apocarotenoid (8) was only obtained from Batch 2 (*ca* 15% of total carotenoid). *19′-Hexanoyloxyfucoxanthin* (2), available in total *ca* 6 mg after repeated purification on TLC (Si gel, 35% Me_2CO in light petroleum = APE); λ_{max}(Me_2CO) 423, 450, (478)nm, % III/II [17] = 20, λ_{max} (MeOH) 443 nm in agreement with fucoxanthin (1); ν_{max} (KBr) 3400 (OH), 3040–2860 (CH), 1930 (allene), 1730 (ester carbonyl), 1660 (conj. ketone), 1610, 1580, 1530, 1460, 1380, 1360, 1250 (ester), 1200, 1160, (tert. OH), 1040 (OH and ester), 970 (*trans* disubst. double bonds), 920, 730 cm^{-1}. The IR-spectrum of fucoxanthin (1), *ex Fucus vesiculosus* was recorded for comparison.

The NMR of (2) and (1) are δ($CDCl_3$).

	Me-16	Me-17	Me-18	Me-19	Me-20,20′	Me-19′
2	0·97	1·04	1·20	(1·92)	1·96	
1	0·97	1·03	1·20	1·92	1·96	1·79

	OCH_2-19′	Me-18′	Me-17′	Me-16′	CH_2-7	CH-8	OAc
2	4·78	1·37	1·10	1·26	2·66*d* 3·63*d*	6·04	2·00
1		1·32	1·07	1·23	2·55*d* 3·60*d*	6·04	2·00

J 18 Hz for CH_2-7 for both (2) and (1). In addition (2) exhibited δ = 8·2 (t?, *ca* 2H, *J* 7 Hz) for —CH_2—$\underline{CH_2}$—CO—O and δ = (0·90) for $\underline{CH_3}$—CH_2?

MS m/e for (2): 772 (M), M-18 = $C_{48}H_{66}O_7$, M-20 = M-2-18?, M-34 = M-18-16?, M-36 = M-18-18?, M-60, M-78 = $C_{46}H_{62}O_5$ = M-18-60?, M-96 = M-60-18-18?, M-110 = M-92-18?, M-114 = $C_{42}H_{58}O_6$, M-116 = $C_{42}H_{56}O_6$, M-132, M-134 = $C_{42}H_{54}O_5$ = M-H_2O-$C_5H_{11}COOH$?, M-150 = M-114-18-18?, M-174 = M-114-60?, M-194 = M-116-60-18?, 536, 358, 326, 311, 237, = $C_{14}H_{21}O_3$, (g) 221 = $C_{14}H_{21}O_2$, (a) 195, 181 = $C_{14}H_{13}$. (2) gave on treatment with *conc.* HCl in ether an intense blue colour.

19′-Hexanoyloxylfucoxanthin 3-acetate (3) prepared by standard acetylation [15] of (2) was less strongly adsorbed (TLC), had unchanged electronic spectrum and *m/e* 814 (M), M-2, M-18, M-36 = M-18-18?, M-78 = M-60-18?, M-96 = M-60-18-18?, M-102 = M-42-42-18?, M-110 = M-92-18?, M-114.

19′-Hexanoyloxyfucoxanthin 3,5′-di(trimethylsilyl) ether (5) prepared by standard silyation [15] of (2) was less strongly adsorbed (TLC) than (3), had unchanged electronic spectrum and *m/e* 916 (M), M-18, M-32, M-60, M-75 = M-60-15?, M-90, M-92, M-108 = M-72-18-18?, M-114, M-116, M-132 = M-114-18?, M-134 = M-116-18?, M-174 = M-114-60?, M-176 = M-116-60?, 293 = silylated (a), 269, 253 = silylated (b).

19′-Hexanoyloxyfucoxanthin 3-acetate-5′-trimethylsilyl ether (4) was prepared by silylation of (3). Silylation was only partial after 1 hr at −30° and 2 hr at 20° and completed after 4 hr at 20°. 5 was less strongly adsorbed (TLC) than (3), had unchanged electronic spectrum and *m/e* 886 (M), M-18 (epoxide) [4].

19′-Hydroxyfucoxanthol (6) was prepared by $LiAlH_4$-reduction of 2 in dry ether. (6) was more strongly adsorbed than (2), had λ_{max} (MeOH) at 408, 420 and 446 nm, % III/II = 86; *m/e* 634 = M = $C_{40}H_{58}O_6$, M-18 = $C_{40}H_{56}O_6$, M-36 = $C_{40}H_{54}O_4$ = M-18-18, M-54 = $C_{46}H_{52}O_3$ = M-18-18-18, M-92 = $C_{33}H_{50}O_6$, M-110 = $C_{33}H_{48}O_5$ = M-92-18, M-116 = $C_{34}H_{46}O_4$, M-128 = $C_{33}H_{46}O_4$ = 92-18-18, M-170 = $C_{30}H_{40}O_4$, M-188 = $C_{30}H_{38}O_3$ = M-170-18, M-200 = $C_{20}H_{38}O_3$, M-206 = $C_{30}H_{36}O_2$ = M-170-18-18, M-224 = $C_{30}H_{34}O$ = M-170-18-18-18, M-236 = $C_{29}H_{34}O$ = M-18-18-200, M-241 = $C_{26}H_{33}O_3$, M-243 = $C_{27}H_{35}O_2$ = M-18-225, M-251 = $C_{25}H_{55}O_3$, M-259 = $C_{26}H_{31}O_2$ = M-18-241, M-261 = $C_{27}H_{33}O$ = M-18-18-225, M-267 = $C_{24}H_{31}O_3$, M-271 = $C_{25}H_{31}O_2$ = M-18-253, 313, 299, 273, 254 = $C_{18}H_{22}O$ (c), 227 = $C_{16}H_{19}O$ (e), 221 = $C_{14}H_{21}O_2$(a), 213 $C_{15}H_{17}O$ (d), 189 = $C_{13}H_{17}O$ (f), 181 = $C_{11}H_{17}O_2$ (b), 167.

19′-hydroxyfucoxanthol 3,8,3′19′*tetraacetate* prepared from (6) was less strongly adsorbed than the latter, had unchanged electronic spectrum and *m/e* 802 = M, M-18, M-42, M-60, M-78 = M-60-18?, M-102 = M-60-42?, M-120 = M-60-60 M-138 = M-60-60-18?, M-162 = M-60-60-42?

19′-hydroxyfucoxanthol 3,8,3′,5′,19′-*penta(trimethylsilyl) ether* prepared by silylation of the tetraacetate was less strongly adsorbed than the latter, had unchanged electronic spectrum and *m/e* 994 (M), M-73, M-90 = M-72-18?, M-160 = M-72-72-18?, M-234 = M-72-72-72-18?

Alkali treatment of 19′-hexanoyloxyfucoxanthin (2). Treatment of (2) in 2% KOH–MeOH for 1 hr caused a hypsochro-

mic shift to 410, 424, 450 nm, % III/II = 100. TLC (kieselgel, 50% APE) showed a strongly adsorbed yellow product turning green–blue. Treatment of (**2**) with 0·1% K_2CO_3 in aq. MeOH for 3 hr followed by transfer to Et_2O and TLC gave a product (**9**?) λ_{max} (Me_2CO) 410, 425, 450 with *m/e* 632 (M?), M-2, M-18, 313, 221, 195, 181.

19-*hexanoyloxyparacentrone* (**8**), available *ca* 1 mg from Batch 2, was less strongly adsorbed than (**2**) had λ_{max}(Me_2CO) 423, 450 (475) nm, % III/II = 20 like **2**; *m/e* 618 (M), M-18, M-18-18, M-52, M-78, M-116 = $C_{33}H_{42}O_4$, M-134 = M-116-18?, 442, 376, 311, (**d** hexanoate?) 284, 232, 211, 195, 161, 149, 125, 119. (**8**) gave no acetate under standard acetylation condition and gave a mono(trimethylsilyl) ether (**9**) on silylation: *m/e* 690 (M). $LiAlH_4$-reduction of (**8**) gave 19-hydroxyparacentrol (**10**) more strongly adsorbed (TLC) than (**8**) λ_{max} (Me_2CO) 398, 423, 450 nm, % III/II = 88. On treatment with 0·1% KOH–MeOH for 4 hr (**8**) was converted to a more polar product (of unchanged chromophore) (**11**?).

Acknowledgements—We are grateful to Dr. H. W. Siegelman, Biology Department, Brookhaven National Laboratory, for providing part of the biological material used. N.A. and W.S. were supported by a research grant from Hoffmann-La Roche, Basel, to S.L.J.

REFERENCES

1. Norgård, S., Svec, W. A., Liaaen-Jensen, S., Jensen, A. and Guillard, R. R. L. (1974) *Biochem. Syst.* **2**, 3.
2. Bonnett, R., Mallams, A. K. Spark, A. A., Tee, J. L., McCormick, A. and Weeden, B. C. L. (1969) *J. Chem. Soc. C*, 429.
3. Jensen, A. (1961) *Acta Chem. Scand.* **15**, 1605.
4. Vetter, W., Englert, G., Rigassi, N. and Schwieter, U. In Isler, O. (Ed.) (1971) *Carotenoids*, Ch. IV, Birkhäuser, Basel.
5. Kjøsen, H. Thesis (1972) Norw. Inst. Technology, Univ. Trondheim.
6. Liaaen, S. and Sørensen, N. A. (1956) *Proc. 2nd Int. Seaweed Symp.*, 25.
7. Nitsche, H. and Egger, K. (1970) *Tetrahedron Letters* 1435.
8. Nitsche, H. (1973) *Z. Naturforsch.* **28c**, 641.
9. Strain, H. H., Svec, W. A., Aitzetmüller, K., Grandolfo, M., Katz, J. J., Kjøsen, H., Norgård, S., Liaaen-Jensen, S., Haxo, F. T., Wegfahrt, P. and Rapoport, H. (1971) *J. Am. Chem. Soc.* **93**, 1823.
10. Johansen, J. E., Svec, W. A. and Liaaen-Jensen, S. (1974) *Phytochemistry* **13**, 2261.
11. Hertzberg, S., Mortensen, T., Siegelman, H. W., Borch, G. and Liaaen-Jensen, S. (To be published).
12. Galasko, G., Haxo, J., Toube, T. P. Weedon, B. C. L., André, D., Barbier, M., Lederer, E. and Villaneuva, V. R. (1969) *J. Chem. Soc. C*, 1264.
13. Hora, J., Toube, T. P. and Weeden, B. C. L. (1970) *J. Chem. Soc. C*, 241.
14. Kjøsen, H. and Liaaen-Jensen, S. (1972) *Acta Chem. Scand.* **26**, 4121.
15. Liaaen-Jensen, S. and Jensen, A. (1971) *Methods Enzymol.* **23**, 586.
16. Siegelman, H. W. and Guillard, R. R. L. (1971) *Methods Enzymol.* **23**, 110.
17. Ke, B., Imsgard, F., Kjøsen, H. and Liaaen-Jensen, S. (1970) *Biochim. Biophys. Acta* **210**, 139.