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THE STRUCTURE OF SIPHONAXANTHIN

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and (in part)

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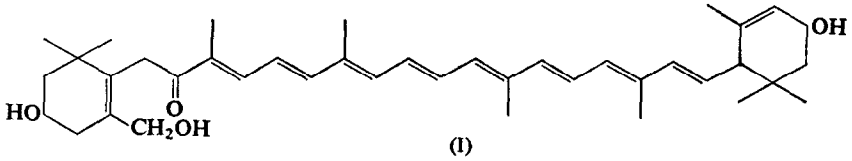
Abstract—The proposed structure of loroxanthin from *Scenedesmus obliquus* has been confirmed by degradation. The structure of siphonaxanthin from *Codium fragile* has been established by NMR and mass spectrometry, and by conversion of siphonaxanthin into loroxanthin. Siphonein, from *C. fragile*, has been shown to be an ester of siphonaxanthin with a dodecenoic acid.

INTRODUCTION

THE PRINCIPAL xanthophylls of most orders of green algae (Chlorophyceae) are identical to those of higher plants,[1-3] but some chlorophycean species contain a further, polar carotenoid, loroxanthin.[4] In addition, members of the order Siphonales contain, as their main xanthophylls, siphonaxanthin and siphonein.[1,3,5] Kleinig and Egger[6] isolated siphonaxanthin from *Caulerpa prolifera* and determined the functional groups present in the molecule, for which they suggested structure I. The position of the primary hydroxyl group, tentatively assigned to the C-5 methyl group, was not conclusively established.



(I)

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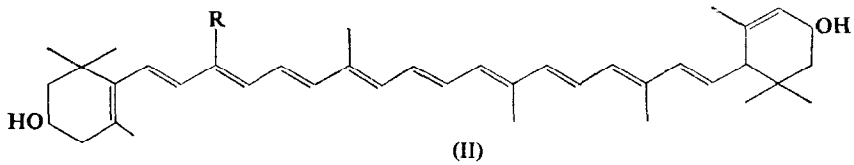
[1] H. H. STRAIN, in *Manual of Phycology* (edited by G. M. SMITH), p. 243, Chronica Botanica, Waltham, Mass. (1951).
[2] H. H. STRAIN, *Annual Priestley Lectures, Penn. State Univ.* **32** (1958).
[3] H. H. STRAIN, in *Biochemistry of Chloroplasts* (edited by T. W. GOODWIN), Vol. I, p. 387, Academic Press, London (1966).
[4] K. ARTZETMÜLLER, H. H. STRAIN, W. A. SVEC, M. GRANDOLFO and J. J. KATZ, *Phytochem.* **8**, 1761 (1969).
[5] H. H. STRAIN, in *Photosynthesis in Plants* (edited by J. FRANCK and W. E. LOOMIS), p. 133, Iowa State College Press, Ames, Iowa (1949).
[6] H. KLEINIG and K. EGGER, *Phytochem.* **6**, 1681 (1967).

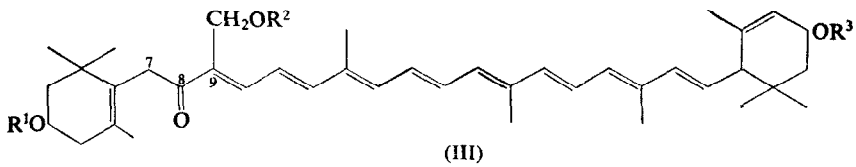
Aitzetmüller *et al.*[4] determined the structure of loroxanthin, and showed it to be a derivative of lutein (IIa) with one of the methyl groups at C-9, 9′, 13 or 13′ modified into a —CH_2OH group. From the mass spectra of loroxanthin and its derivatives, it was concluded that the primary hydroxyl group was probably located on the methyl group at C-9, loroxanthin thus having structure IIb. The location of the primary hydroxyl group, however, was not rigorously proved.



(II)

(a: R = CH_3) (b: R = CH_2OH)

Recently, Kleinig, Nitsche and Egger[7] reported the conversion of siphonaxanthin into a product with chromatographic properties and a visible spectrum identical to those of loroxanthin. This led them to propose the modified structure, IIIa, for siphonaxanthin.



(III)

(a: $R^1 = R^2 = R^3 = H$) (b: $R^1 = H, R^2 = R^3 = CH_3$)

(c: $R^1 = COCH_3, R^2 = R^3 = CH_3$) (d: $R^1 = R^2 = R^3 = COCH_3$)

In none of these investigations, however, was the location of the primary hydroxyl group conclusively established. We have therefore confirmed the structure of loroxanthin (from *Scenedesmus obliquus*) by degradation. We have also confirmed the structure of siphonaxanthin (from *Codium fragile*) by NMR and mass spectrometry, and by conversion of siphonaxanthin into loroxanthin. The structure of siphonein, an ester of siphonaxanthin, has also been investigated.

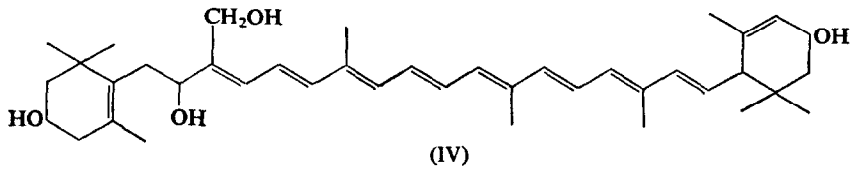
RESULTS AND DISCUSSION

Siphonaxanthin, isolated from *Codium fragile* and purified in a range of column and TLC systems, had a rounded visible spectrum characteristic of a keto-carotenoid, with an ill-defined λ_{max} at *ca.* 448 nm, somewhat lower than the previously reported value (455 nm).[5,6] Reduction with $NaBH_4$ caused a hypsochromic shift of 27 nm as expected for reduction of an oxo- group in conjugation with the main chromophore. The product, siphonaxanthol, had a sharp visible spectrum with λ_{max} at 397, 421, 450 nm, in agreement with previously reported values, and indicative of a conjugated octaene chromophore.

The mass spectrum of siphonaxanthin showed the parent ion, M^+ at *m/e* 600 ($C_{40}H_{56}O_4$) and fragment ions at *m/e* 582, 564 and 546 (weak) due to respective losses of 1, 2 and 3 molecules of water. The mass spectrum of siphonaxanthin acetate showed the parent ion at *m/e* 726·4495 ($C_{46}H_{62}O_7$), and fragment ions at *m/e* 666 (M-60, metastable at *m/e* 611; $666^2/726 =$

[7] H. KLEINIG, H. NITSCHE and K. EGGER, *Tetrahedron Letters* **59**, 5139 (1969).

611·0), 606 (M-60-60, metastable at *m/e* 551; $606^2/666 = 551{\cdot}4$) and 546 (weak, M-60-60-60), due to losses of 1, 2, and 3 molecules of acetic acid respectively. Siphonaxanthin thus has three primary or secondary hydroxyl groups.



(IV)

The mass spectrum of siphonaxanthol (IV) had the parent ion M^+ at *m/e* 602 ($C_{40}H_{58}O_4$) and fragment ions at *m/e* 584, 566, 548 and 530 (weak), produced by respective losses of 1, 2, 3 and 4 molecules of water, thus confirming the reduction of one carbonyl group in siphonaxanthin to an alcohol. Siphonaxanthol acetate had mol. wt. 770 ($C_{48}H_{66}O_8$) and the mass spectrum showed fragment ions at *m/e* 710, 650, 590 and 530 (weak) confirming its tetraacetate nature.

Treatment of siphonaxanthin with methanolic HCl gave three products, the major, least polar, one being identified as a siphonaxanthin dimethyl ether (IIIb). The mass spectrum of this compound indicated a mol. wt. of 628 ($C_{42}H_{60}O_4$) and had major fragment ions at *m/e* 596 (M—CH_3OH, metastable at *m/e* 566; $596^2/628 = 565{\cdot}6$) and 564 (M—$2CH_3OH$, metastable at *m/e* 534; $564^2/596 = 533{\cdot}7$). A weak peak at *m/e* 610 (M—H_2O) was also observed. Formation of a dimethyl ether under these conditions shows that two of the hydroxyl groups in siphonaxanthin are allylic.

A feature of the mass spectra of the dimethyl ether and its acetate (IIIc) (mol. wt. 670, $C_{44}H_{62}O_5$) was the presence of fragment ions at *m/e* 572 (metastable at *m/e* 521); $572^2/628 = 521{\cdot}0$) and 614 (metastable at *m/e* 563; $614^2/670 = 562{\cdot}7$) respectively, due to loss of 56 mass units by the *retro*-Diels–Alder fragmentation characteristic of the α-ionone end-group.[8,9] Strong peaks were also observed at *m/e* 476 (metastable at *m/e* 361; $476^2/628 = 360{\cdot}8$) for siphonaxanthin dimethyl ether, and at *m/e* 518 (metastable at *m/e* 400·5; $518^2/670 = 400{\cdot}5$) for the acetate. This loss of 152 mass units from the parent ion is similar to that observed in the mass spectra of lutein-3′-*O*-methyl ether and its acetate, and is due to cleavage of the C-6′,7′ bond.[10] Siphonaxanthin, therefore, like lutein, contains a 3′-hydroxylated α-ionone ring. In the mass spectrum of siphonaxanthin, fragment ions were observed at *m/e* 544 and 462, corresponding to losses of 56 and 138 mass units from the parent ion, in agreement with the presence of this α-ionone ring.[10]

The electronic spectrum of siphonaxanthin showed the presence of a conjugated system of eight C=C double bonds and one carbonyl group, the latter probably being located at C-8 or C-8′, as in the case of fucoxanthin.[11] This was supported by the mass spectra of siphonaxanthin and siphonaxanthin dimethyl ether, and their acetates, which all exhibited fragment ions produced by cleavage of the bonds α- to the carbonyl group. The composition of several of these fragment ions was confirmed by high resolution mass spectrometry.

[8] U. SCHWIETER, H. R. BOLLIGER, L. H. CHOPARD-DIT-JEAN, G. ENGLERT, M. KOFLER, A. KÖNIG, C. VON PLANTA, R. RÜEGG, W. VETTER and O. ISLER, *Chimia* **19**, 294 (1965).
[9] B. C. L. WEEDON, *Chem. Brit.* **3**, 424 (1967).
[10] C. R. ENZELL, G. W. FRANCIS and S. L. JENSEN, *Acta Chem. Scand.* **23**, 727 (1969).
[11] R. BONNETT, A. K. MALLAMS, A. A. SPARK, J. L. TEE, B. C. L. WEEDON and A. MCCORMICK, *J. Chem. Soc.* C 429 (1969).

In the case of siphonaxanthin, cleavage of the C-7,8 bond gave fragment ions at *m/e* 447 ($C_{30}H_{39}O_3$, M-153), 429 (M-153—H_2O, metastable at *m/e* 412; $429^2/447 = 411{\cdot}7$), and 411 (M-153—$2H_2O$, metastable at *m/e* 394; $411^2/429 = 393{\cdot}8$), and at *m/e* 355, 341, 337, 323, 319, 305 due to loss of toluene (92) or xylene (106) from these fragments.[8] Cleavage of the C-8,9 bond gave fragment ions at *m/e* 419 ($C_{29}H_{39}O_2$, M-181), 401 (M-181—H_2O) and 383 (M-181—$2H_2O$), and at *m/e* 327, 313, 309, 295, 291 and 277 due to losses of toluene and xylene from these fragments.

Siphonaxanthin triacetate (IIId) gave similar fragment ions at *m/e* 531 ($C_{34}H_{43}O_5$, M-195), 471 (M-195—CH_3COOH, metastable at *m/e* 418; $471^2/531 = 417{\cdot}8$) and 411 (M-195—$2CH_3COOH$, metastable at *m/e* 359; $411^2/471 = 358{\cdot}6$) by cleavage of the C-7,8 bond and at *m/e* 503 ($C_{33}H_{43}O_4$, M-223), 443 (M-223—CH_3COOH, metastable at *m/e* 390; $443^2/503 = 390{\cdot}2$) and 383 (M-223—$2CH_3COOH$, metastable at *m/e* 331; $383^2/443 = 331{\cdot}1$), by cleavage of the C-8,9 bond. Peaks due to losses of toluene or xylene from these ions were also observed.

Cleavage of the C-7,8 and C-8,9 bonds of siphonaxanthin dimethyl ether also resulted in the loss of 153 and 181 mass units respectively, as for siphonaxanthin. Fragment ions due to multiple losses of 153 or 181 mass units together with one or two molecules of methanol (32 or 64) and toluene (92) or xylene (106) were also observed. The basic fragments lost by the acetate of siphonaxanthin dimethyl ether were of 195 and 223 mass units, as in the case of siphonaxanthin acetate. Again these fragmentations were confirmed by the presence of ions formed by multiple losses of 195 or 223 and methanol, toluene or xylene.

It is apparent from this that the fragment ions obtained by cleavage of the C-7,8 and C-8,9 bonds of siphonaxanthin and derivatives are polyenic and retain the two allylic hydroxy-methoxy- or acetoxy-substituents, and therefore the α-ionone ring. The neutral species lost contain only the non-allylic hydroxy- or acetoxy-substituent. Since the α-ionone ring has only one allylic hydroxyl substituent, the second allylic hydroxyl group must be located in the polyene chain, probably by replacement of a methyl group by CH_2OH. This is further supported by fragmentations observed in the mass spectrum of siphonaxanthin dimethyl ether, which indicate losses of 136 mass units, corresponding to a methoxy-derivative of *m*-xylene, as observed by Aitzetmüller *et al.*[4] in the mass spectrum of loroxanthin dimethyl ether.

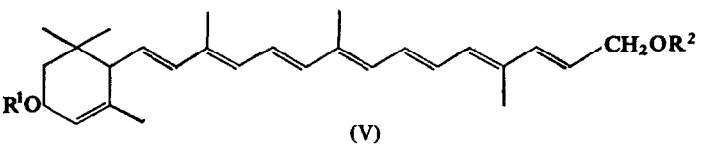
In the NMR spectrum of siphonaxanthin, signals at 9·06 and 9·17 τ and 8·39 τ are assigned to the protons of the methyl groups at C-1′ and C-5′ respectively of the α-ionone ring, and the 6-proton signal at 9·01 τ to the *gem*-methyl groups at C-1 of the other ring, in agreement with its formulation as a non-allylic 3-hydroxy-β-ionone ring. Signals at 8·04 τ (6 protons) and 8·10 τ (3 protons) are assigned to the "in-chain" (C-9,9′,13,13′) methyl groups. The slight shift in the signal of one of these methyl groups is similar to that reported for loroxanthin.[4] These signals, however, accounted for only nine protons, or three methyl groups. A 2-proton signal at 5·54 τ was attributable to the methylene protons of a —CH_2OH group at one of the C-9 or C-13 positions, as described for loroxanthin.[4] A signal at 8·52 τ (3 protons) is attributed to the C-5 methyl group, the high value presumably being due to positive shielding by the C-8 carbonyl group in close proximity. The signal for the C-1 methyl groups is also at somewhat higher field than in the case of lutein. A signal at 6·52 τ was assigned to the C-7 methylene group, in contrast to the doublets at 6·40 and 7·45 τ in the spectrum of fucoxanthin.

The electronic, mass and NMR spectral data thus indicate that siphonaxanthin has the carbon skeleton proposed by Kleinig and Egger,[6] with hydroxyl groups at C-3,3′, and a third,

primary, allylic hydroxyl group on one of the C-9,13 methyl groups. The location of this primary hydroxyl group was therefore investigated.

Allylic dehydration of siphonaxanthol gave a product, mol. wt. 584. The mass spectra of this compound and its acetate were identical to those quoted by Aitzetmüller *et al.*[4] for loroxanthin and its acetate. Loroxanthin was therefore isolated from *Scenedesmus obliquus* and the two samples compared directly. The mass spectra and chromatographic properties of the two samples and their acetates were identical. Because of isomerization under the acidic conditions of the dehydration reaction, the visible spectra could not be compared directly, but the electronic spectra of the iodine isomerization products were identical. This indicated that the primary hydroxyl group of siphonaxanthin was in the same position as that of loroxanthin. This was provisionally located by Aitzetmüller *et al.*[4] at C-9, and confirmation of this was sought by degradation.

Loroxanthin triacetate was treated with $KMnO_4$ under conditions similar to those used for the preparation of α- and β-citraurin acetates from lutein and zeaxanthin.[12]



(V)

(a: $R^1 = R^2 = H$) (b: $R^1 = R^2 = CH_3$)

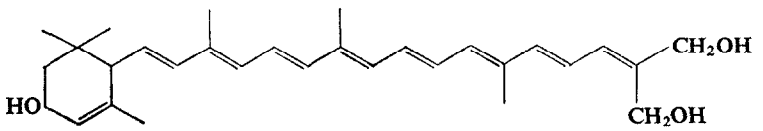
The complex mixture of apocarotenals was separated by TLC and the electronic spectra of the main components determined. The main products were reduced with $NaBH_4$ to the corresponding apocarotenols, saponified, and the electronic and mass spectra determined. These products included a compound with λ_{max} 375, 395, 419 nm, formed from an apocarotenal with λ_{max} 441 nm. This compound had mol. wt. 394 ($C_{27}H_{38}O_2$), and the mass spectrum had fragment ions at *m/e* 376 (M—H_2O, metastable at *m/e* 359; $376^2/394 = 358{\cdot}8$), 358 (M—$2H_2O$), 363 (M—CH_2OH, metastable at *m/e* 334; $363^2/394 = 334{\cdot}4$), and 345 (M—CH_2OH—H_2O, metastable at *m/e* 316·5; $345^2/363 = 316{\cdot}6$). This product was therefore a dihydroxy compound, and a fragment ion at *m/e* 256 (M-138, metastable at *m/e* 166; $256^2/394 = 166{\cdot}3$) indicated the presence of a 3-hydroxy α-ionone ring, and the probable structure Va. This was confirmed by formation of a dimethyl ether with methanolic HCl, showing that both hydroxyl groups were allylic. The mass spectrum of the dimethyl ether had the parent ion, M^+ at *m/e* 422 ($C_{29}H_{42}O_2$) and fragment ions at *m/e* 390 (M—CH_3OH, metastable at *m/e* 360·5; $390^2/422 = 360{\cdot}4$) and 358 (weak, M—$2CH_3OH$). Fragment ions at *m/e* 366 (M-56, metastable at *m/e* 317·5; $366^2/422 = 317{\cdot}4$) and 270 (M-152, metastable at *m/e* 173; $270^2/422 = 172{\cdot}7$) confirmed the presence of a 3-methoxy α-ionone ring, and hence structure Vb, for the dimethyl ether.

This degradation product had therefore retained the α-ionone ring and the methyl groups at C-9′,13′ and 13. The primary hydroxyl group of loroxanthin was not retained in this product, and must therefore be located on the C-9 methyl group, as proposed by Aitzetmüller *et al.*[4]

Additional confirmation of this assignment was obtained from the mass spectra of other reduced and saponified degradation products. One of these products had λ_{max} 396, 419, 446 nm, and mol. wt. 450 ($C_{30}H_{42}O_3$). The mass spectrum contained fragment ions at *m/e*

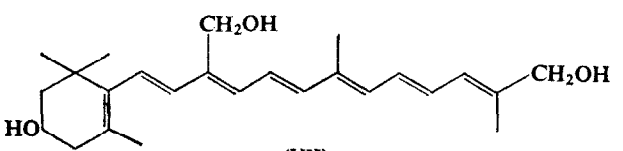
[12] P. Karrer, H. Koenig and U. Solmssen, *Helv. Chim. Acta* **21**, 445 (1938).

432, 414, 396 formed by respective loss of 1, 2 and 3 water molecules, and fragment ions at *m/e* 312 (M-138, metastable at *m/e* 216; $312^2/450 = 216{\cdot}3$) and 294 (M—H_2O-138, metastable at *m/e* 200; $294^2/432 = 200{\cdot}0$) indicated the presence of the 3-hydroxy α-ionone ring. This compound, assigned structure VI, has retained the methyl groups at C-9′, 13′, 13 and the —CH_2OH group at C-9.



(VI)

A third product was obtained, with λ_{max} 377, 393 nm, and mol. wt. 384 ($C_{25}H_{36}O_3$). Fragment ions at *m/e* 366 (M-18), 348 (M-36) and 330 (weak, M-54) indicated the presence of three hydroxyl groups. Other major fragment ions were observed at *m/e* 353 (M—CH_2OH, metastable at *m/e* 324·5; $353^2/384 = 324{\cdot}5$), 335 (M—CH_2OH—H_2O, metastable at *m/e* 318; $335^2/353 = 317{\cdot}9$) and 317 (weak M—CH_2OH—$2H_2O$). No fragment ions were observed at *m/e* 246 (M-138) or 228 (M-18-138). This product had therefore retained the primary hydroxyl group and the 3-hydroxy β-ionone ring of loroxanthin (structure VII).



(VII)

The identification of these degradation products unequivocally establishes that the CH_2OH group of loroxanthin is located at C-9, and confirms the structure of loroxanthin as IIb.

Insufficient material was available to permit similar degradation of siphonaxanthin, but conversion of siphonaxanthin into loroxanthin established that the primary hydroxyl group of siphonaxanthin is in the same position as that of loroxanthin, i.e. at C-9. Siphonaxanthin thus has structure IIIa as postulated by Kleinig *et al.*[7]

Siphonein

In unsaponified extracts of *Codium fragile*, most of the siphonaxanthin occurs in the form of an ester, siphonein, siphonaxanthin being liberated by saponification. The spectral max. of siphonein, however (456 nm), is, like that of siphonaxanthin triacetate, somewhat higher than that of siphonaxanthin (448 nm). The electronic spectra of the $NaBH_4$ reduction products of these compounds however are identical. This was interpreted by Kleinig and Egger[6] as an effect of hydrogen bonding between the —CH_2OH and >C=O groups in siphonaxanthin, which could not occur in the acetate. This also indicated that the primary hydroxy group of siphonaxanthin is esterified in siphonein. Our results are in agreement with this.

It was also shown that in siphonein from *Caulerpa prolifera*, the esterifying acid was lauric acid (dodecanoic acid). The mass spectrum of siphonein from *Codium fragile* showed a weak parent ion, M^+, at *m/e* 780 ($C_{52}H_{76}O_5$) and strong fragment ions at *m/e* 762 (M—H_2O, metastable at *m/e* 745; $762^2/780 = 744{\cdot}4$), 582 (M-198) and 564 (M—H_2O-198, metastable

at m/e 417; $564^2/762 = 417{\cdot}4$). Mol. wt. and the loss of 198 mass units due to cleavage of the allylic ester link indicate that in *Codium fragile* the esterifying fatty acid is not lauric acid but a dodecenoic acid. This has not yet been further characterized.

EXPERIMENTAL

Electronic spectra were recorded in ethanol. Mass spectra were determined in an A.E.I. MS12 instrument at an ion source temperature of 240°. High resolution mass spectra and NMR spectra in $CDCl_3$ at 100 mHz were determined by the Physico-Chemical Measurements Unit, Harwell, Berks.

Biological Materials

Codium fragile was gathered at Woods Hole, Mass., U.S.A., and at Stony Brook, Long Island, N.Y., U.S.A. *Scenedesmus obliquus* (Strain D3, kindly provided by Dr. R. Powls) was cultured, in collaboration with Dr. Powls, at 30°, in the dark for 7 days on a nitrate medium supplemented with 0·5% glucose and 0·25% yeast extract.[13]

Isolation and Purification of Siphonaxanthin

Codium fragile was homogenized in a Waring Blendor and the lipid material then extracted with acetone, transferred to ether, and the ether removed *in vacuo*. When siphonein was not required, the extract was cold saponified, by the usual technique.[14] The lipid material (after saponification if required) was chromatographed in 20% (v/v) E^*/P on a column of neutral alumina (Brockmann Grade III), and elution was continued with increasing amounts of E in P. After elution with 70% (v/v) E/P to remove less polar materials, siphonein was eluted with 100% E, and siphonaxanthin with 5% EtOH/E. These fractions were evaporated to dryness. Siphonaxanthin and siphonein were further purified by TLC (Table 1).

TABLE 1. TLC SYSTEMS USED FOR PURIFICATION OF SIPHONAXANTHIN, LOROXANTHIN AND DERIVATIVES

Compound	Solvents used		
	TLC 1 (Silica gel G)	TLC 2 (MgO–Kieselgur G)	TLC 3 (Silica gel G)
Siphonaxanthin	5% EtOH/E*	45% A/P	2% EtOH/E
Siphonaxanthin triacetate	70% E/P	15% A/P	50% E/P
Siphonein	70% E/P	25% A/P	E
Siphonaxanthol	7% EtOH/E	40% A/P	5% EtOH/E
Siphonein diacetate	70% E/P	15% A/P	50% E/P
Siphonaxanthol tetracetate	70% E/P	20% A/P	50% E/P
Siphonaxanthol dehydration product	1% EtOH/E	40% A/P	4% EtOH/E
Siphonaxanthol dehydration product—acetate	22% E/P	10% A/P	30% E/P
Siphonaxanthin dimethyl ether	30% E/P	30% A/P	E
Siphonaxanthin dimethyl ether acetate	30% E/P	20% A/P	40% E/P
Loroxanthin	1% EtOH/E	40% A/P	4% EtOH/E
Loroxanthin triacetate	22% E/P	10% A/P	30% E/P
Loroxanthin dimethyl ether	70% E/P	20% A/P	30% E/P
Loroxanthin dimethyl ether acetate	30% E/P	15% A/P	40% E/P

* Abbreviations: EtOH = ethanol, E = diethyl ether, A = acetone, P = light petroleum, b.p. 40–60°.

Isolation and Purification of Loroxanthin

Scenedesmus obliquus was harvested by centrifugation and the cells disrupted in a French press. The cell debris was collected and the lipid material extracted with acetone, transferred to ether, and saponified, in the usual way.[14] The saponified material was chromatographed, in 20% E/P, on a column of neutral alumina (Brockmann Grade III). After elution of unwanted substances with 70% E/P, loroxanthin was eluted with 5% EtOH/E, and further purified by TLC (Table 1).

* Abbreviations: E = diethyl ether, P = light petroleum (b.p. 40–60°), A = acetone, EtOH = ethanol.

[13] E. KESSLER, W. ARTHUR and J. E. BRUGGER, *Arch. Biochem. Biophys.* **71**, 326 (1957).
[14] G. BRITTON and T. W. GOODWIN, in *Methods in Enzymology* (in press).

Acetylation

Pigments were acetylated with Ac_2O in pyridine in the normal way,[14] and the products were purified by TLC (Table 1).

Allylic Methylation[4]

Compounds were treated with a 1% solution of conc. HCl in methanol for 15 min. The products were transferred to ether and the HCl removed by washing with saturated $NaHCO_3$ solution (twice) and water (twice). The ethereal solution was evaporated to dryness and the products separated by TLC (Table 1).

Allylic Dehydration[15]

Siphonaxanthol (IV, 0·2 mg) was treated with 0·03 M HCl in $CHCl_3$ for 15 min in daylight. The $CHCl_3$ solution was washed free from acid with saturated $NaHCO_3$ (twice) and water (twice), and evaporated to dryness. The dehydration product was purified by TLC (Table 1).

Reduction with Sodium Borohydride[14]

Compounds, dissolved in ethanol, were treated with $NaBH_4$ and the course of the reaction followed spectrophotometrically. On completion of preparative-scale reductions, the products were transferred to ether, the ethereal solutions washed with water and evaporated, and the products purified by TLC (Table 1).

Permanganate Oxidation of Loroxanthin Triacetate[12]

Loroxanthin triacetate (18 mg) was dissolved in benzene (7·5 ml) and 7·5 ml of an aq. solution of $KMnO_4$ (72 mg) and Na_2CO_3 (108 mg) added. The mixture was shaken, in the dark, for 105 min at 20°, and the resulting suspension extracted with ether (5 × 30 ml). The ethereal solution was washed twice with water and evaporated to dryness. The residue was chromatographed on thin layers (0·5 mm) of Silica gel G with 45% *E/P* as developing solvent. Two main bands were obtained: band A, R_f 0·5 and band B, R_f 0·3. Band A was further separated by TLC on MgO–Kieselgur G (1:1) with 15% *A/P* as developing solvent, and gave two bands, A_1 (R_f 0·7) and A_2 (R_f 0.2).

Fraction A_1 (λ_{max} 441 nm) was reduced with $NaBH_4$, and the product saponified. The saponification product was purified by TLC on Silica gel G in ether to give the apocarotenol Va (R_f 0·8, λ_{max} 375, 395, 419 nm). A sample of this compound was converted into the dimethyl ether, Vb. The amounts of fraction A_2 were too small to permit further analysis.

Band B was separated by TLC on MgO–Kieselgur G (1:1) with 20% *A/P* as developing solvent, into two bands, B_1 (R_f 0·4) and B_2 (R_f 0·2). Fraction B_1 (λ_{max} 420 nm) was reduced and saponified and the product purified by TLC on Silica gel G in ether to give the apocarotenol, VII (R_f 0·6, λ_{max} 378, 394 nm). Fraction B_2 (λ_{max} 450 nm) was reduced and saponified and the product purified by TLC on Silica gel G, with ether, to give the apocarotenol VI (R_f 0·2, λ_{max} 396, 418, 445 nm).

The mass spectra of apocarotenols Va, VI and VII, and the dimethyl ether, Vb, were determined, and the salient features are discussed in the text.

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