

## Bacterial Carotenoids

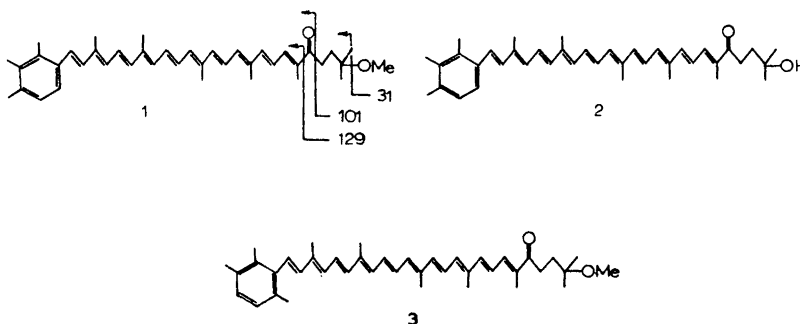
XXXVII.\* Carotenoids of Thiorhodaceae 9. Structural Elucidation of Five Minor Carotenoids from *Thiothece gelatinosa*

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The structures of five conjugated carbonyl carotenoids (4, 7, 11, 17, 15) were determined from spectroscopic evidence (electronic, IR, and mass spectra) and chemical transformations. Each minor carotenoid has one methoxylated terminus identical to the acyclic end of okenone (1) and the difference in structural characteristics arises from variations in the terminus corresponding to the aryl end of okenone. Included in these new structures are two apo-carotenoids (15, 17) and the first aromatic methyl carotenoate (7).

The isolation, morphological and growth character of *Thiothece gelatinosa* strain 2611 has previously been described.<sup>1</sup> The same study included an analysis of the carotenoid composition. *Thiothece* strain 2611 is characterized by a high carotenoid content of which okenone (1) is the major pigment.

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In addition, four minor carotenoids designated *Thiothece*-478, -484, -OH-484, and -polar-484 were isolated. Of this latter group *Thiothece*-OH-484 was identified as a demethylated okenone (2) on the basis of co-chromatography test and electronic spectrum, and structure 3 tentatively considered for *Thiothece*-478.

A re-investigation of the minor carotenoids of *Thiothece* strain 2611 has been undertaken in an attempt to unambiguously assign structures to the minor components already mentioned and perhaps detect other pigments whose structures might indicate the biosynthetic scheme which culminates in okenone (1).

### RESULTS AND DISCUSSION

The carotenoids isolated in the present study from *Thiothece* strain 2611 constituted 1.71 % of dry cell weight. Of this pigment mixture, the vast majority was okenone but in addition five conjugated carbonyl carotenoids were isolated (Table 1).

Table 1. Carotenoid composition and yield of *Thiothece* strain 2611.

Carotenoid <sup>a</sup>	% of total	Content in mg
<i>Thiothece</i> -474 <sup>b</sup> (4)	1.15	0.424
Okenone (1)	93.7	34.600
<i>Thiothece</i> -484 (7)	0.95	0.350
<i>Thiothece</i> -425 (17)	0.16	0.070
<i>Thiothece</i> -460 (15)	0.27	0.100
<i>Thiothece</i> -OH-484 (11)	4.05	1.500

<sup>a</sup> Increasing adsorbance on deactivated Al<sub>2</sub>O<sub>3</sub>.

<sup>b</sup> Corresponds to *Thiothece*-478.<sup>1</sup>

#### *Thiothece*-474

*Thiothece*-474 exhibited two *cis-trans* isomers separable on kieselguhr paper (Table 2). The electronic spectrum of the *trans* isomer showed considerable loss in fine structure in shifting from petroleum ether to methanol solvent indicative of a conjugated carbonyl function.<sup>2</sup> Strong IR absorption at 1655 cm<sup>-1</sup> confirmed the presence of a conjugated carbonyl group. Medium intensity absorption at 1080 cm<sup>-1</sup> suggested a methoxy function<sup>3</sup> while absorption around 800 cm<sup>-1</sup> found in aromatic carotenoids<sup>4,5</sup> was absent (Fig. 1). The mass spectrum exhibited the molecular ion at *m/e* 582 consistent with the formula C<sub>41</sub>H<sub>58</sub>O<sub>2</sub>. Losses of 92, 106, and 158 mass units<sup>6</sup> confirmed this assignment. Fragment ions of diagnostic importance were observed at *m/e* 550, 481, and 453 attributed to the loss of methanol, C<sub>6</sub>H<sub>13</sub>O, and C<sub>7</sub>H<sub>13</sub>O<sub>2</sub>. These same fragments arise from the acyclic terminus of okenone (1)<sup>6</sup> and suggest that okenone and *Thiothece*-474 have one common methoxylated end group. Additional important ions were observed at *m/e* 353 and 339 due

to combined losses of (92+137) and (106+137) mass units. The loss of 137 mass units ( $C_{10}H_{17}$ ) is observed in carotenoids possessing unsubstituted  $\beta$ -end groups from rupture of the 7,8 double bond. This specific fragmentation has been found to be prominent in cleavages involving the  $\beta$ -end group and takes preference over in-chain cleavages.<sup>6</sup> That the loss of 137 mass units is not observed from the molecular ion may be explained by selected fragmentation of the acyclic terminus in monocyclic carotenoids.<sup>6</sup>

The spectral data are compatible with a 1'-methoxy-1',2'-dihydro- $\beta$ ,  $\psi$ -caroten-4'-one (by new nomenclature<sup>7</sup>) formulation for *Thiothece*-474 (4).

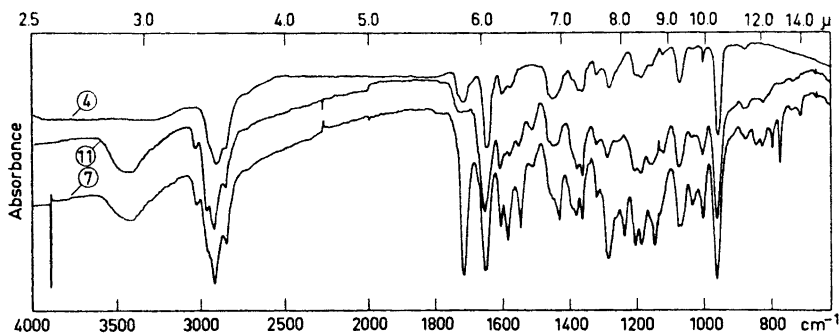
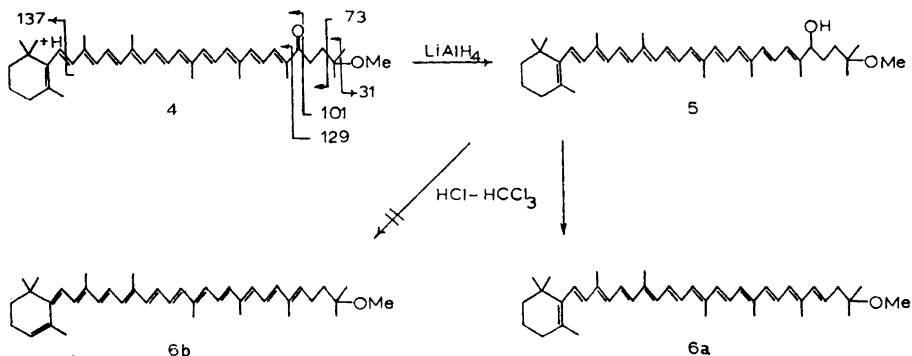


Fig. 1. Infrared spectra (KBr) of *Thiothece*-474 (4), *Thiothece*-484 (7) and *Thiothece*-OH-484 (11).

Further evidence supporting structure (4) comes from chemical studies. Hydride reduction of *Thiothece*-474 gave the allylic alcohol (5) in virtually quantitative yield. Chromatographic behaviour confirmed the formation of a mono-ol (Table 3). The reduction product exhibited electronic spectrum indistinguishable from synthetic  $\beta$ ,  $\psi$ -carotene ( $\gamma$ -carotene by old nomenclature), and the hypsochromic shift (13 nm) caused by the reduction supported the location of the original carbonyl group at the aliphatic end of the chromophore.<sup>8</sup> The mass spectrum of the reduced compound (5) showed the molecular



ion at  $m/e$  584 which is expected for reduction of only one carbonyl group;  $M-18$  ( $H_2O$ ),  $M-92$ , and  $M-106$  peaks.

Treatment of **5** with  $HCl-HCCl_3$ <sup>9</sup> gave a major product with an extended chromophore. The allylic dehydration products **6a** (plectanixanthin<sup>10</sup> chromophore) and **6b** (4,4'-didehydro- $\beta,\alpha$ -carotene<sup>11</sup> chromophore) may be expected, of which only **6a** was obtained judged by the electronic spectrum.

*Thiothece*-474 (**4**) is considered to be identical with *Thiothece*-478. The previously suggested structure (**3**)<sup>1</sup> is thus revised.

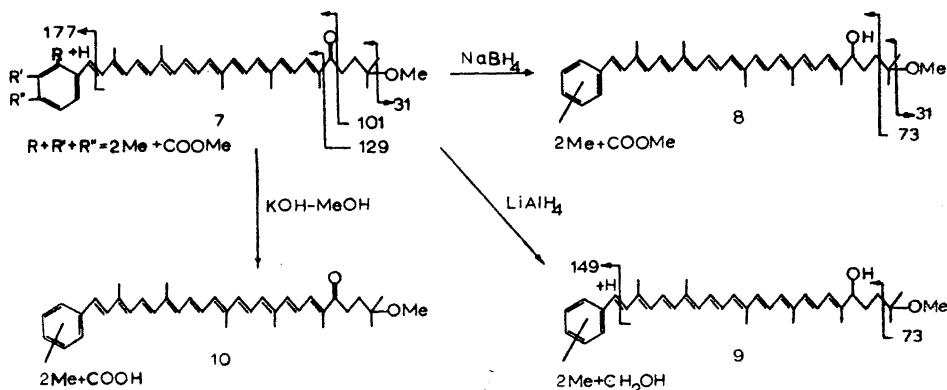
#### *Thiothece*-484

*Thiothece*-484, isolated in the amount of 350  $\mu g$ , had two *cis-trans* isomers (Table 2). The electronic spectrum of the *trans* isomer in petroleum ether was indistinguishable from that of okenone (**1**),<sup>12</sup> but in acetone the maximum showed slight bathochromic displacement relative to **1**.

IR absorption (Fig. 1) occurred at  $1655\text{ cm}^{-1}$  (conjugated carbonyl),  $1080\text{ cm}^{-1}$  (methoxy group),  $1720\text{ cm}^{-1}$ ,  $1290\text{ cm}^{-1}$ , and  $1150\text{ cm}^{-1}$  (possibly due to an aryl ester<sup>11</sup>) and at  $800\text{ cm}^{-1}$  (two adjacent aromatic hydrogens<sup>3</sup>).

High precision mass spectrometry showed the molecular ion at  $m/e$  622.4012 consistent with the formula  $C_{42}H_{54}O_4$  (calc. 622.4022), confirmed by  $M-92$ ,  $M-106$ , and  $M-158$  ions.<sup>6</sup> Ions at  $M-32$ ,  $M-101$ , and  $M-129$  established that *Thiothece*-484 has an acyclic terminus common to okenone (**1**).<sup>6</sup> Fragment ion at  $m/e$  177.0908 ( $C_{11}H_{13}O_2$ , calc. 177.0915) could not be accounted for by in-chain cleavage at the acyclic carbonyl end. This latter fragment could arise from rupture of the 7,8-bond of a trimethyl aryl end group in which one methyl was replaced by a carbomethoxy group.

Selective reduction with  $NaBH_4$  caused a hypsochromic shift of 13 nm and gave a single product (**8**) with chromatographic behaviour indicative of a mono-ol (Table 3); confirmed by mass spectrometric determination of the molecular weight ( $m/e$  624 =  $M$ ). Lithium aluminium hydride reduction of *Thiothece*-484 resulted in a product (**9**) much more polar than the borohydride product. The absorption maximum showed 2 nm hypsochromic displacement relative to that of the borohydride product, demonstrating the small contribu-



tion of the second carbonyl function to the chromophore in *Thiothece*-484. The mass spectrum of the  $\text{LiAlH}_4$  reduction product (9) gave the molecular ion at  $m/e$  596 ( $\text{C}_{41}\text{H}_{56}\text{O}_3$ ) confirming the reduction of both a carbonyl and a carbomethoxy group. A fragment ion at  $m/e$  149 corresponded to the  $m/e$  177 ion previously mentioned for the parent compound (7). Structure 7 is proposed for *Thiothece*-484 and 8 and 9 for the borohydride and lithium hydride reduction products. The electronic spectrum of 9 corresponds to that of okenol<sup>4,12</sup> (hydride reduced okenone); hence, only one of the  $\alpha$ -positions to the polyene chain can be substituted. Since the IR spectrum reveals the presence of two adjacent aryl hydrogens ( $800\text{ cm}^{-1}$ ) the aryl group of *Thiothece*-484 must be 1,2,3-trisubstituted analogous to the situation in okenone (1).<sup>4</sup> Unambiguous assignment of the position of the carbomethoxy group cannot be made from the available data. However, 3-position is tentatively preferred since 1- or 2-position on the aromatic ring would result in steric hindrance from both *ortho* substituents and esters of this configuration are notably difficult to hydrolyze.<sup>13-15</sup> The degree to which alkaline hydrolysis of *Thiothece*-484 proceeded was not suggestive of a strongly hindered ester.

#### *Thiothece*-OH-484

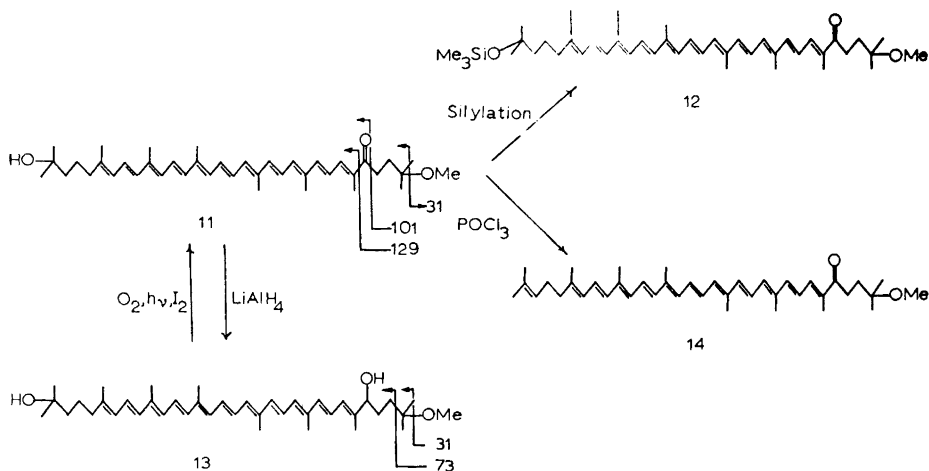
*Thiothece*-OH-484 crystallized from petroleum ether-acetone solution melted at  $143^\circ$ . After cursory examination, a demethylated okenone structure (2) was suggested for this pigment.<sup>1</sup> However, mass spectrometry gave the molecular ion at  $m/e$  600 (corresponding to  $\text{C}_{41}\text{H}_{60}\text{O}_3$ ) incompatible with structure 2 which requires a molecular weight of 564. In addition to fragment ions due to the loss of toluene ( $M-92$ ), xylene ( $M-106$ ) and dimethylcyclo-decapentaene ( $M-158$ ),<sup>6</sup> peaks were observed at  $M-18$  ( $\text{H}_2\text{O}$ ),  $M-101$  ( $\text{C}_6\text{H}_{13}\text{O}$ ),  $M-129$  ( $\text{C}_7\text{H}_{13}\text{O}_2$ ), and  $M-32$  ( $\text{CH}_3\text{OH}$ ). The latter three fragments, now familiar, strongly implicate the presence of the acyclic terminus of okenone and the appearance of  $M-18$  suggests that this entity must originate from the other end of the carotenoid.

The IR spectrum of *Thiothece*-OH-484 confirmed the presence of a conjugated carbonyl group and a methoxy function. Only a weak hydroxy band was apparent at  $1160\text{ cm}^{-1}$  at the frequency of tertiary hydroxy groups<sup>3</sup> (Fig. 1).

The electronic spectrum of *Thiothece*-OH-484 closely resembles that of okenone (1) and on changing the solvent displayed the properties of a conjugated carbonyl carotenoid.<sup>2</sup> Since one terminus of *Thiothece*-OH-484 is structurally identical to the acyclic end of okenone (1), the opposing end must make the same contribution to the chromophore as the trimethyl aryl ring of okenone, which experience has demonstrated to be close to one aliphatic double bond.<sup>12</sup> Thus *Thiothece*-OH-484 must, in order to accommodate the established molecular weight and absorption spectra, have two acyclic end groups.

Attempted acetylation of *Thiothece*-OH-484 was negative but the trimethylsilyl ether was smoothly formed, establishing the tertiary nature of the hydroxy group.<sup>16</sup> Mass spectrometry showed the molecular ion at  $m/e$  672 in agreement with the formula  $\text{C}_{44}\text{H}_{68}\text{O}_3\text{Si}$ .

Structure *11* is proposed for *Thiothece*-OH-484 and *12* for the silylated derivative.



Hydride reduction of *11* gave a single product *13* with chromatographic properties typical of a diol (Table 3) and with electronic spectrum characteristic of an aliphatic undecaene chromophore; superimposable on that of lycopene. Compound *13* was easily converted back to the parent compound (*12*) by air oxidation in the presence of light and  $I_2$ .<sup>17</sup> The mass spectrum of *13* gave the molecular ion at  $m/e$  602 ( $C_{41}H_{62}O_3$ ) confirming reduction of a single carbonyl group. Careful treatment of *11* with  $POCl_3$  in pyridine<sup>18</sup> gave a major product (*14*) which on comparative chromatography established the loss of one hydroxyl group (Table 3).

Formally *Thiothece*-OH-484 is 1'-hydroxy-1-methoxy-1,2,1',2'-tetrahydro- $\psi$ , $\psi$ -caroten-4-one.

#### *Thiothece*-460

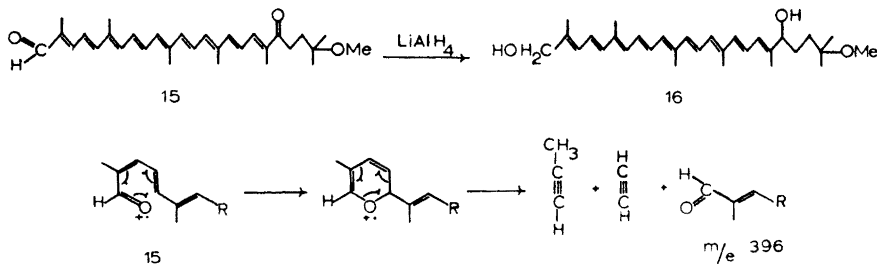
*Thiothece*-460, 100  $\mu$ g, had absorption maxima at 460.5 nm in petroleum ether and 473.5 nm in methanol; the spectrum in the latter solvent showed loss of fine structure. Apparent also is the large shift of 13 nm in the absorption maximum in shifting from the non-polar to polar solvent; a feature not observed in the other carotenoids of *Thiothece*-2611.

The IR spectrum of *Thiothece*-460 showed a broad carbonyl absorption between 1640–1680  $cm^{-1}$  the position and shape of which suggested the presence of two different carbonyl groups. A band at 1085  $cm^{-1}$  indicated the presence of a methoxy group.

Mass spectrometry revealed the molecular ion at  $m/e$  462 ( $C_{31}H_{42}O_3$ ). Loss of 92 and 158 mass units was not observed but loss of 106 mass units was significant. The same situation has been reported for bixindial.<sup>6</sup> Fragment ions at  $M-32$  and  $M-129$  again suggest that one end of *Thiothece*-460 resembles the acyclic end of okenone (*1*). Additional ions were observed at  $m/e$  422 ( $M-40$ ) and  $m/e$  396 ( $M-66$ ).

Reduction of *Thiothece*-460 with  $\text{LiAlH}_4$  gave a product whose chromatographic behaviour was indicative of a diol (Table 3). The mass spectrum of the reduction product gave the molecular ion at  $m/e$  466, confirming reduction of two carbonyl functions. The electronic spectrum of reduced *Thiothece*-460 had maximum at 432.5 (*cis-trans* mixture) nm indicative of an aliphatic nonaene chromophore.

*Thiothece*-460 probably has the apo-carotenal structure proposed in formula 15 and the hydride reduction product 16. A possible rationalization of the fragment ion  $M - 66$  ( $40 + 26$ ) is shown.



Structure 15 is consistent with the chromatographic behaviour of *Thiothece*-460 (Table 2) and with the electronic spectrum. The spectral effect in visible light of two carbonyl groups in conjugated  $\omega, \omega'$ -systems (15) is not additive. In aliphatic carotenoids the spectral contribution of the second carbonyl is in the order of 1–7 nm.<sup>19</sup> In *Thiothece*-460 with a nonaene polyene chain the spectral contribution from both carbonyl groups is 22 nm. The spectral contribution of the keto group in okenone, *Thiothece*-474 and *Thiothece*-484, is *ca.* 14 nm. Assuming the same contribution in *Thiothece*-460, the second carbonyl would cause a bathochromic shift of *ca.* 8 nm in agreement with that found for other  $\omega, \omega'$ -dicarbonyl polyenes.<sup>19</sup>

Formally *Thiothece*-460 is 1-methoxy-4-oxo-1,2-dihydro-8'-apo- $\psi, \psi$ -caroten-8-al.

#### *Thiothece*-425

*Thiothece*-425 was the least abundant of the minor pigments. The electronic spectrum was similar to that of *Thiothece*-460 but was hypsochromically displaced (Table 2).

On alumina and silica gel *Thiothece*-425 was slightly less polar than *Thiothece*-460 (Tables 1 and 2). The mass spectrum showed the molecular ion at  $m/e$  396 compatible with the formula  $\text{C}_{26}\text{H}_{36}\text{O}_3$ . Hydride reduction gave a single product with absorption maximum at 390 nm (*cis-trans* mixture) suggestive of a heptaene chromophore and with chromatographic properties of a diol (Table 3).

*Thiothece*-425 is probably 17 and its reduction product 18.

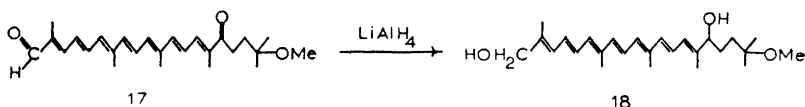


Table 2. Absorption maxima in visible light in various solvents and chromatographic data of the minor carotenoids isolated from *Thiothece* strain 2611 and their *cis* isomers.

Carotenoid	Stereoisomer	$R_F$ value <sup>a</sup>			Absorption maxima in nm in			petroleum ether <sup>c</sup>
		2 % <sup>b</sup>	5 %	10 %	acetone	methanol		
<i>Thiothece</i> -474 (4)	<i>trans</i>	0.43			(450) 477 (504)	477	448 474 500	
	Neo A	0.69			(445) 471.5 (500) 368	474	(445) 471 (495)	360
<i>Thiothece</i> -484 (7)	<i>trans</i>		0.11	0.27	(465) 487.5 518	(462) 486 (511)	458 484 513.5	
	Neo A		0.21	0.55	(455) 478.5 (510) 380	(455) 477 (506)	450 473.5 506	
	Neo B		0.30	0.67	(455) 479 (508) 378	478	375 450 474 504	
<i>Thiothece</i> -OH-484 (11)	<i>trans</i>		0.10	0.23	460 484.5 515	(460) 483 (510)	459 484 515	
	Neo A		0.19	0.38	(459) 480 (508)		373 455 480 511.5	
	Neo B		0.24	0.50	(455) 477.5 (505)		373.5 455.5 477.5 507	
	Neo C		0.28	0.56	(455) 475 (500)		373 452 475 (503)	
<i>Thiothece</i> -460 (15)	<i>trans</i>		0.12		(445) 471 506	(445) 473.5 (500)	435 460.5 494.5	
	Neo A		0.18		438 463.5 (500)		(394) 430 455.5 488	
	Neo B		0.26		(412) 436 461		(403) 425 452.5 (488)	
<i>Thiothece</i> -425 (17)	<i>trans</i> (+ <i>cis</i> )		0.13		413 434 461	437 460	402 425 452.5	

<sup>a</sup> Schleicher & Schüll No. 287 kieselguhr paper.

<sup>b</sup> Acetone in petroleum ether.

<sup>c</sup> B.p. 40–60°C.



By the new nomenclature 17 is 1-methoxy-1,2-dihydro-12'-apo- $\psi$ , $\psi$ -caroten-12'-al.

### CONCLUSION

The minor carotenoids encountered, *Thiothece*-474 (4) and possibly also *Thiothece*-OH-484 (11), may represent biochemical intermediates of okenone (1). It is assumed, but not experimentally proved that the biosynthesis of 1,2,3-trimethyl aryl end groups occurs *via*  $\beta$ -ring intermediates.<sup>20</sup>

The three other new carotenoids (7, 15, 17) encountered may be considered as metabolic products of okenone (1) produced by further biochemical oxidation of an aromatic methyl group (7) or by oxidative cleavage of the polyene chain to form apo-carotenals (15, 17).

### EXPERIMENTAL

*Biological material.* *Thiothece* strain 2611 of the Institute of Microbiology, the University of Göttingen, was used.

*Cultivation.* The cultivation was carried out in Göttingen under the supervision of Professor N. Pfennig by the method previously used;<sup>1</sup> yield 30 g wet cells.

Table 3. Absorption maxima in visible light and  $R_F$  values of carotenoid derivatives studied.

Carotenoid	$R_F$ value on silica gel <sup>a,b</sup>				Absorption maxima in nm in						
	2 % <sup>b</sup>	5 %	10 %	15 %	acetone			petroleum ether <sup>d</sup>			
<i>Thiothece</i> -474 (4)	0.08	0.29	0.34	0.47	(450)	477	(504)	(448)	474	500	
LiAlH <sub>4</sub> reduced (5)	0	0.05	0.13	0.22	438	461.5	491.5	(410)	433	457	487
LiAlH <sub>4</sub> reduced and HCl/CHCl <sub>3</sub> dehydrated (6)	0.28	0.59	0.84	1.0	448	473	504 <sup>c</sup>				
<i>Thiothece</i> -484 (7)		0.06	0.187	0.33	(465)	487.5	518		458	484	513.5
LiAlH <sub>4</sub> reduced (9)		0	0	0.04	447	472.5	503				
NaBH <sub>4</sub> reduced (8)		0	0.05	0.17	460	474.5	504		445	471.5	500
Saponified (10)		0	0	0	460	487.5	518				
<i>Thiothece</i> -OH-484 (11)		0	0.14	0.23	460	484.5	515		459	484	515
LiAlH <sub>4</sub> reduced (13)		0	0.05	0.10	445.5	483.5	504				
POCl <sub>3</sub> dehydrated (14)		0.10	0.50	0.70	456	484	514 <sup>c</sup>				
TMS ether (12)		0.10	0.55	0.73	459	487.5	515				
<i>Thiothece</i> -460 (15)		0.04	0.13	0.27	(445)	471	506	(412)	435	460.5	494.5
LiAlH <sub>4</sub> reduced (16)		0	0	0.06	414.5	436.5	466.5 <sup>c</sup>		410.5	432.5	462.5 <sup>c</sup>
<i>Thiothece</i> -425 (17)		0.05	0.15	0.31	413	434	461		402	425	452.5
LiAlH <sub>4</sub> reduced (18)		0	0	0.07	371	394	418.5 <sup>c</sup>		367	390	414.5 <sup>c</sup>

<sup>a</sup> 1 mm thick layers.

<sup>b</sup> Acetone in petroleum ether.

<sup>c</sup> *cis-trans* Mixture.

<sup>d</sup> B.p. 40–60°C.

*Materials and methods.* Instrumentation, pigment extraction, saponification and chromatography were as generally used in this laboratory.<sup>21-23</sup> After column chromatography on Woelm neutral alumina activity grade 3, the individual carotenoids were further purified by TLC (silica gel HF-254) using acetone-petroleum ether mixtures as developer.

#### *Thiothece-474 (4)*

*Characterization.* Absorption maxima in several solvents are given in Table 2 and the IR spectrum in Fig. 1. The mass spectrum had peaks in the upper region at  $m/e$  582 (M), M-15, M-31, M-32 (metastable peak at  $m/e$  520), M-73, M-92 (metastable peak at  $m/e$  413), M-101, M-106, M-129, M-158, M-92-129, M-106-129, M-106-137.

*Lithium aluminium hydride reduction to 5.* 4 (100  $\mu$ g) in dry ether was quantitatively reduced in 30 sec with lithium aluminium hydride to 5; pigment yields were around 90%. 5 thus produced, normally consisted of a *cis-trans* mixture; for characterization of the *trans* isomer see Table 2. The mass spectrum showed peaks in the upper mass region at  $m/e$  584 (M), M-18, M-91, M-92 (metastable peak at  $m/e$  415), M-106, M-92-18, M-106-18, M-158, M-106-18-73, and M-106-92-73.

*Dehydration with acid chloroform to 6.*<sup>24</sup> 5 (30  $\mu$ g) in dry ethanol-free  $\text{CHCl}_3$  (1 ml) was treated with 0.2 N dry HCl in  $\text{CHCl}_3$  (0.1 ml) for 10 min. *Ca.* 70% of 5 was converted to 6; for electronic spectrum and  $R_F$  value see Table 3.

#### *Thiothece-484 (7)*

*Characterization.* Spectral data in visible light are given in Table 2 and the IR spectrum in Fig. 1. The mass spectrum had peaks at  $m/e$  622.4012 (M), M-31, M-32 (metastable peak at  $m/e$  559), M-79, M-92 (metastable peak at  $m/e$  453), M-101, M-106, M-129, M-31-106, M-158, M-129-106, and  $m/e$  177.0908.

*Sodium borohydride reduction to 8.* 7 (50  $\mu$ g) in methanol (2 ml) was quantitatively reduced with excess hydride for 5 min. Spectral data (visible light) of 8 are given in Table 3 along with the chromatographic behaviour on silica gel plates. The mass spectrum had peaks at  $m/e$  624 (M), M-18, M-31, M-32 (metastable peak at  $m/e$  558), M-18-32, M-73, M-92, M-106, M-18-92, M-18-106, and  $m/e$  177.

*Lithium aluminium hydride reduction to 9.* Treatment of 7 (50  $\mu$ g) with excess lithium aluminium hydride in dry ether resulted in the quantitative formation of 9. Spectral data are given in Table 3 and Fig. 2. Peaks in the mass spectrum were observed at  $m/e$  596 (M), M-18, M-73, M-18-73, M-92, M-106, M-18-92, M-18-106, and  $m/e$  149.

*Saponification to 10.* 7 in 10% methanolic potassium hydroxide (2 ml) was stirred at 20° for 18 h. The solution was acidified with solid ammonium chloride and the pigment extracted into ether. Thick layer chromatography on silica gel showed two bands, one identical to starting material and the second with  $R_F=0$ . The latter band was extracted and on partition test with ether and alkaline methanol-water remained in the hypophase. Acidification of the hypophase made the pigment epiphasic. *Trans* 10 was isolated by paper chromatography ( $R_F=0.2$  on kieselguhr paper, 30% acetone in petroleum ether) and had spectral characteristics in visible light identical to those of 7. On silica gel plates 7 had  $R_F$  value of 0.04 in acetone-petroleum ether (40:60).

#### *Thiothece-OH-484 (11)*

*Characterization.* Spectral data are given in Table 3 (visible light) and Fig. 2 (IR), respectively. Peaks in the mass spectrum appeared at  $m/e$  600 (M), M-15, M-18, M-32 (metastable peak at  $m/e$  539), M-18-32, M-92 (metastable peak at  $m/e$  430), M-101, M-106, M-18-106, M-129 and M-158.

*Trimethylsilyl ether 12.* 11 (40  $\mu$ g) in dry pyridine (1 ml) was reacted with hexamethyldisilazane (0.4 ml) and chlortrimethylsilane (0.2 ml)<sup>16</sup> for 15 min. Chromatography on silica gel gave 12 whose visible spectra and chromatographic characteristics are given in Table 3.

*Acetylation.* Attempted acetylation of *11* in the usual manner<sup>22</sup> was negative.

*Lithium aluminium hydride reduction to 13.* *11* reduced in the normal manner gave *13* in 95 % yield; for spectral properties in visible light see Table 3. Prominent ions in the mass spectrum of *13* occurred at  $m/e$  602 (M), M-18, M-32, M-36, M-18-32, M-91, M-92 (metastable peak at  $m/e$  432), M-106, M-18-106 and M-158.

*Dehydration with POCl<sub>3</sub> to 14.* To *11* (30  $\mu$ g) in dry pyridine (3 ml) was added POCl<sub>3</sub> (0.1 ml) during 5 min with rapid stirring. After 15 min the pigment was extracted into ether and *14* isolated by silica gel chromatography in 30 % yield.

#### *Thiothece-460 (15)*

Chromatographic behaviour and spectral data are given in Table 3. The mass spectrum of *15* had peaks at  $m/e$  462 (M), M-15, M-31, M-32 (metastable peak at  $m/e$  422), M-40, M-66, M-106, and M-129. The IR spectrum had bands at 2820  $\text{cm}^{-1}$  (C-H), 1640-1680  $\text{cm}^{-1}$  (conjugated carbonyl), 1080  $\text{cm}^{-1}$  (OMe), and 970  $\text{cm}^{-1}$  (*trans* disubstituted double bonds). Lithium aluminium hydride reduction of *15* in dry ether for 30 sec gave *16*. Spectral and chromatographic data for *16* are given in Table 3.

#### *Thiothece-425 (17)*

Spectral and chromatographic data are given in Table 3. The mass spectrum of *17* showed a distinct molecular ion at  $m/e$  396 but few fragment ions in the upper mass region. Lithium aluminium hydride reduction of *17* in the normal manner gave *18*; for spectral and chromatographic data see Table 3.

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