## The Structure of Vaucheriaxanthin

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Allenic xanthophyll, position of in-chain substitution

Vaucheriaxanthin is 19'-OH and not 19-OH neoxanthin. This structural conclusion was borne out by mass, PMR and infrared (IR) spectra from the free pigment and its derivatives (vaucheriaxanthal, vaucheriaxanthal-diacetate, vaucheriaxanthal-diacetate-mono(trimethylsilyl)-ether, vaucheriaxanthintriacetate- and triacetate-monosilanate, vaucheriaxanthin-dimethylether) and by comparison of the characteristic lines of epoxi-xanthophylls at m/e 181 and m/e 221 in the mass spectrum.

To vaucheriaxanthin the structure of a 5',6'-epoxi-6,7-didehydro-5,6,5',6'-tetrahydro- $\beta$ , $\beta$ -carotene-3,5,19,3'-tetrol has been assigned <sup>1</sup>. The position of the primary OH has been derived from absorbance changes of 20 nm between vaucheriaxanthal and its furanoic derivative. In a reinvestigation of loroxanthin (19-OH lutein), which is identical with pyrenoxanthin <sup>2</sup>, a spectral difference of 20 nm has been found between loroxanthalepoxide (oxidated with monoperphthalic acid resp. *p*-chloroanil) and its furanoic derivative, too.

## Results and Discussion

Support for the fixation of the primary OH near the epoxide ring (Fig. 1 A, 1) comes from the PMR spectrum. Signals attributable to methyl-protons were found at 0.98, 1.04, 1.16, 1.21, 1.35, 1.78 and 1.96 ppm, with relative intensities of 1:1:1:1:

Fig. 1. Formulae and assignments of the PMR signals of vaucheriaxanthin (A) and neoxanthin (B).

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2:1:2. The corresponding resonances of neoxanthin (Fig. 1B) were at 0.96, 1.06, 1.14, 1.17, 1.33, 1.79, 1.91 and 1.94 ppm, with intensities of 1:1:1: 1:2:1:1:2. A signal for the  $C_9$  methyl (1.78 ppm) was present, whereas a resonance at 1.91 ppm ( $C_{9'}$ methyl) was completely absent. Instead there was a peak at 4.34 ppm indicating (as in loroxanthin and siphonaxanthin  $^{2-5}$ ) the in-chain substitution. In the olefinic region a singlet at 6.00 ppm could be assigned to an allenic proton as in neoxanthin, mimulaxanthin (a diallenic xanthophyll  $^6$ ) and peridinin, a pigment from dinoflagellates  $^7$ . This allene group was readily detected in the IR, Fig. 2, at 1923 cm $^{-1}$ .

Vaucheriaxanthin ( $\lambda_{max}$  466, 436 and 418 in ethanol) had mol.wt. 616 (C<sub>40</sub>H<sub>56</sub>O<sub>5</sub>), Fig. 3. Results obtained from  $C_{7.7^{'}}$  and  $C_{15,15^{'}}$  deuterated carotenoids or synthetic analogues of  $\beta$ -carotene, where the  $C_{13,13}$  methyl groups had been shifted to  $C_{14,14}$  , 8, 9 have shown toluene (92 m.u.) to be derived exclusively from the 10-10' and xylene (106 m.u.) from the 8-8' region. Hence in neoxanthin, Fig. 4, strong lines were observed for M-92, M-110 = M-92-18, M-125 = M-92-18-15, M-128 = M-92-18-18 and M-143 = M-92-18-18-15. (Loss of  $CH_3 = 15$  m.u. was also found in the M-51 = M-18-18-15 ion.) M-106 is very reduced. M-92, M-110 and M-128 were also found in the vaucheriaxanthin-spectrum, but instead of M-125, M-123 = M-92-18-13 and instead of M-143, M-141 = M-92-18-1318-18-13 were present. This additional loss of 13 m.u. (CH) is also responsible for the intense lines at M-49 = M-18-18-13, M-67 = M-18-18-18-13 and M-85 = M-18-18-18-13 (with the intensity of the M-92 line). Additional peaks represent M-122 and M-140. M-122 can be explained as M-m-methylbenzylalcohol and has also been observed in the

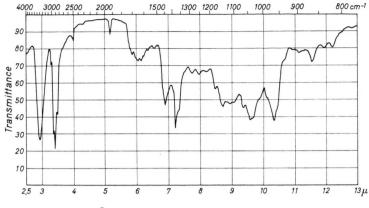


Fig. 2. IR spectrum of vaucheriaxanthin in KBr.  $\,$ 

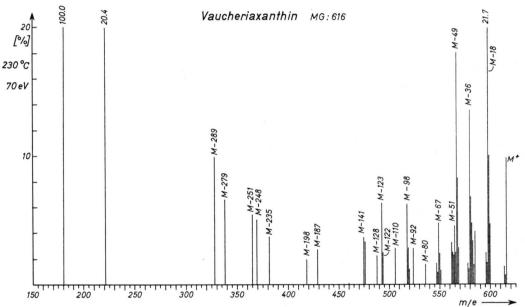


Fig. 3. Mass spectrum of vaucheriaxanthin.

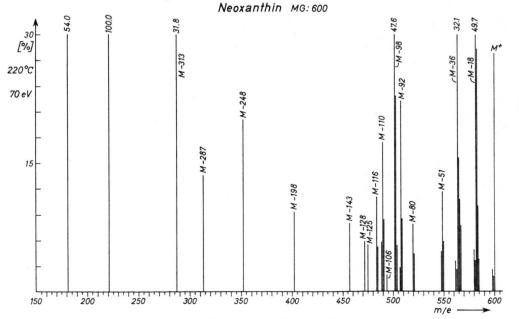


Fig. 4. Mass spectrum of neoxanthin.

ment) 2.

MS of loroxanthin <sup>3</sup>, M-140 is M-122-18. Carotenoid-epoxides show losses of 80 m.u. These and the ions produced by concerted loss of water (M-98, M-116) are readily detected in the neoxanthin-spectrum, whereas in vaucheriaxanthin only M-80 and M-98 are abundant. The mechanism for the formation of the M-80 fragment is not yet clear. According to Bonnett et al. <sup>10</sup> it is formed as shown in Fig. 5 A. Not in agreement with this proposal is, that in fucochrome -8-d (neochrome -8-d) 81 m.u. are eliminated <sup>11</sup>. Therefore an other mechanism has been proposed, Fig. 5 B. If this scheme were correct, a substitution of the C<sub>9</sub> methyl should prevent the formation of the M-80 fragment and M-94

Fig. 5. Possible mechanisms of the formation of the M-80 fragment from xanthophyll-epoxides. A, according to Bonnett <sup>10</sup>. B, according to Budzikiewicz <sup>11</sup>.

diepoxide, loroxanthalepoxide (no line at M-94)

and loroxanthinepoxidetriacetate (no M-138 frag-

Vaucheriaxanthin yielded a triacetate with mol. wt. 742 (C<sub>46</sub>H<sub>62</sub>O<sub>8</sub>), Fig. 1 A, **2**, Fig. 6. Besides strong lines resulting from elimination of acetates (M-60, M-120) and acetate plus H<sub>2</sub>O (M-78, M-138) the corresponding ions of toluene elimination were found (M-92; M-152 = M-92-60; M-170 = M-92-60-18). As in the free pigment M-106 nearly completely is absent. This is the same with M-164, corresponding to M-m-methylbenzylacetate (which has been found in loroxanthin- and loroxanthinepoxidetriacetate, too <sup>2, 3</sup>). But in the lower mass region a strong ion at m/e 410 (M-332) can

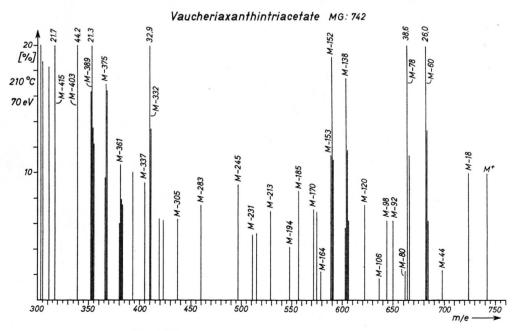


Fig. 6. Mass spectrum of vaucheriaxanthin-triacetate.

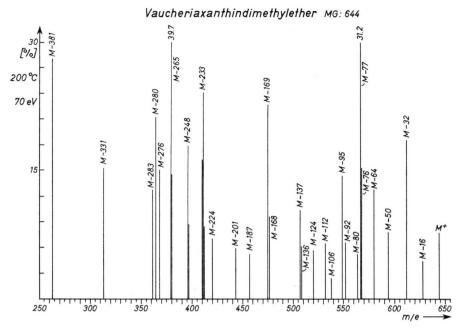


Fig. 7. Mass spectrum of vaucheriaxanthin-dimethylether.

Table I. Mass spectra of vaucheriaxanthal-diacetate mol.wt. 698 (A) and vaucheriaxanthin-triacetatemono(trimethylsilyl)ether mol.wt. 814 (B) at 230 °C and 70 eV. Intensities in relation to the base peak (BP) = R.I.

	A	М — R.I.	$0=\mathbf{M}^+$	34 (18-16)		47 (18-16-13)		60	78		
			8.4	6.6			1.9		2.4	9.7	
	$\mathbf{A}$	M -	80	92	93	98	106	110	121	138	
		R.I.	4.6	6.0	11.2	19.1	2.3	3.1	3.9	3	
	$\mathbf{A}$	м —	148	149	151		153	173	176	177	
		R.I.	7.1	17	9		6.7	4.6	6	13.5	
	$\mathbf{A}$	M -	185	277	295		317	330	343	409 (BP)	
		R.I.	5	6.5	5.4		8.4	7.3	32	100	
	В	м —	56	72	90		132	150	164	170	
		R.I.	1	8.8	7.8		10	16.6	3	2.6	
	$\mathbf{B}$	M -	192	206	210		224	242	266	317	
		R.I.	4.4	5	6.2		3.3	2.6	3	3.3	
	В	M -	355	377	404		447	461	475	640 (BP)	
		R.I.	2.8	2.8	9.6		8.6	9	24.3	100	

be explained as M-164-92-60-16, which is accompanied by a fragment at m/e 368 (M-374 = M-332-42), produced by additional elimination of ketene (42 m.u.) from one acetyl-group.

In contrast to the triacetate, the triacetatemonosilanate (mol.wt. 814,  $C_{49}H_{70}O_8Si$ , Fig. 1 A, 3, Table I) had a line at M-164, which was accompanied by one at M-224 = M-164-60. M-92 and M-106 were completely absent, as was the mol-peak. Strong lines were at M-72 = M-56-16, M-132 = M-60-56-16, M-150 = M-90-60, M-170 = M-90-80, M-192 = M-60-60-56-16, M-206 = M-90-60-56,

M-210=M-90-60-60 and M-226=M-60-60-60-56-16. M-56 ( $C_4H_8$ ) itself was also present. Its origine is not yet clear.

Oxidation of the free pigment with p-chloroanil yielded a red coloured aldehyde,  $\lambda_{\rm max}$  468 nm (468 – 436 = 32 nm), which after acid treatment absorbed at 448 nm (468 – 448 = 20 nm). With loroxanthalepoxide in relation to loroxanthinepoxide a shift of 27 nm has been observed and the furanoic loroxanthalepoxide absorbed 20 nm at shorter wavelengths, too <sup>2</sup>. These close spectral relationships and the fact, that the aldehyde merely gave a diacetate

and this still a mono (trimethylsilyl) ether support the localization of the primary OH to the end of the conjugated system. Vaucheriaxanthaldiacetate had mol.wt. 698 (C<sub>44</sub>H<sub>58</sub>O<sub>7</sub>). Strong lines (Table I) were at M-18 (tert OH), M-34 (tert OH + epoxide), M-78 = M-60-18, M-93 = M-80-13, M-98 =M-80-18, M-149 = M-80-2  $\times$  15-3  $\times$  13, M-153 = M-80-60-13,  $M-177 = M-80-3 \times 15-4 \times 13$ , M-185M-92-80-13,  $M-277 = M-2 \times 92-80-13$  and  $M-295 = M-2 \times 92-80-18-13$ . An ion corresponding to M-m-tolualdehyde (M-120) was not found, but instead lines at M-138 = M-120-18, M-148 = M-120-15-13, M-151 = M-120-18-13 and M-176= M-120-18-15-13 were found.

From the vaucheriaxanthindimethylester (Fig. 1 A, 4) mol.wt. 644 ( $C_{42}H_{56}O_5$ ) intense ions are produced by losses of methanol (M-32, M-64), methanol plus H<sub>2</sub>O (M-50) and methanol plus toluene (M-124). As in the free pigment and the vaucheriaxanthaldiacetate 13 m.u. are eliminated, leading to lines at M-77 = M-32-32-13, M-95 = M-32-32-18-13, M-137 = M-124-13 and M-169 =M-92-32-32-13. An ion at M-136 as in loroxanthindimethylether 2, 3 can be formulated as M-m-methylbenzylether. The low intensity may be ascribed to the competitive elimination-reaction of 80 m.u. More intense lines from this fragment, produced by additional losses of methanol, can be found as M-168 = M-136-32, M-248 = M-136-80-32 and M-280 =M-136-80-32-32, Fig. 7.

 $\begin{array}{lll} {\rm R_1}{\rm =}{\rm R_2}{\rm =}{\rm H}\;;\;\;{\rm R_3}{\rm =}{\rm CH_3} & m/e\;181\;m/e\;221\\ {\rm R_1}{\rm =}{\rm R_2}{\rm =}{\rm H}\;;\;\;{\rm R_3}{\rm =}{\rm CH_2}{\rm OH} & m/e\;181\;m/e\;237 \end{array}$ 

 $\begin{array}{l} {\rm R_1 \! = \! R_2 \! = \! CH_3CO};\;{\rm R_3 \! = \! CH_2COOCH_3} \quad m/e\;223\;\;m/e\;305 \\ {\rm R_1 \! = \! R_2 \! = \! CH_3CO};\;{\rm R_3 \! = \! CHO} \quad m/e\;223\;\;m/e\;277 \end{array}$ 

5  $R_1 = R_2 = H$ ;  $R_3 = CH_2OCH_3$  m/e 181 m/e 251

Fig. 8. Assignments of the m/e 181 and m/e 221 ions of vaucheriaxanthin and its derivatives.

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Additional support for the localization of the primary OH comes from the lower mass region. All hitherto examined xanthophylls (not fucoxanthin) show intense ions at m/e 181 and m/e 221  $^{10-12}$ , which were formulated in Fig. 8. In the MS the line at m/e 221 always is more intense than that at m/e181. E. g. in antheraxanthin the ratio of m/e 181: m/e 221 is 1:4, in neoxanthin 1:2 and in the diepoxide violaxanthin 1:2. Substitution of the methyl near the epoxide reverses this relation. So in loroxanthinepoxide ( $R_1$ : OH,  $R_2$ : CH<sub>2</sub>OH) m/e 181: m/e 237 is 3:1 (no line at m/e 221), in loroxanthalepoxide it is 5:1 (m/e 181:m/e 235), and in loroxanthinepoxide-triacetate m/e 223: m/e 305 (no line at 181 and 221) is 9:12. These results were obtained from vaucheriaxanthin and its derivatives, too. Vaucheriaxanthin gave (Fig. 8, 2) 6:1, the triacetate (3) 8:1, vaucheriaxanthaldiacetate (4) 2.5:1, vaucheriaxanthin-dimethylether (5) 6:1 and the vaucheriaxanthintriacetate-monosilylether (3) 10:1. Similar results have been obtained from the MS of the allenic xanthophyll peridinin 7, where the  $C_{g'}$  methyl is replaced by the carbonyl of a buteno-

As vaucheriaxanthin occurs together with neoxanthin, the elucidation of their biosynthetic connections is a quite urgent problem. This is the same with the in-chain hydroxilated xanthophylls loroxanthin and siphonaxanthin, which probably originate from lutein.

## **Material and Methods**

All important data are described in detail elsewhere  $^{2, 13-15}$ .

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