

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- β,β -carotene-3,5,19,3'-tetrol has been assigned¹. 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 pyrenoxanthin², 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, I) 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:

2:1:2. The corresponding resonances of neoxanthin (Fig. 1 B) 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 C₉ methyl (1.78 ppm) was present, whereas a resonance at 1.91 ppm (C₉-methyl) was completely absent. Instead there was a peak at 4.34 ppm indicating (as in loroxanthin and siphonaxanthin²⁻⁵) 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 xanthophyll⁶) and peridin, a pigment from dinoflagellates⁷. This allene group was readily detected in the IR, Fig. 2, at 1923 cm⁻¹.

Vaucheriaxanthin (λ_{\max} 466, 436 and 418 in ethanol) had mol.wt. 616 (C₄₀H₅₆O₅), Fig. 3. Results obtained from C_{7,7'} and C_{15,15'} deuterated carotenoids or synthetic analogues of β -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 CH₃ = 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-92-18-18-13 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-18-18-18-18-13 (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

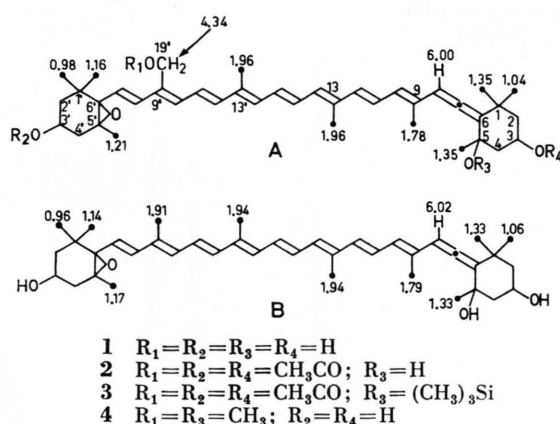


Fig. 1. Formulae and assignments of the PMR signals of vaucheriaxanthin (A) and neoxanthin (B).

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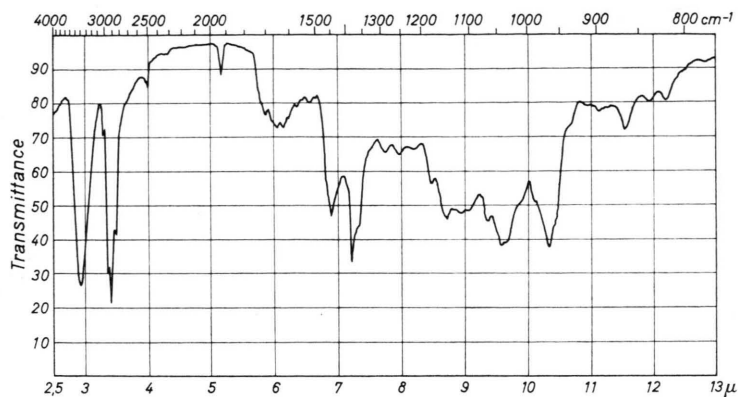


Fig. 2. IR spectrum of vaucherixanthin in KBr.

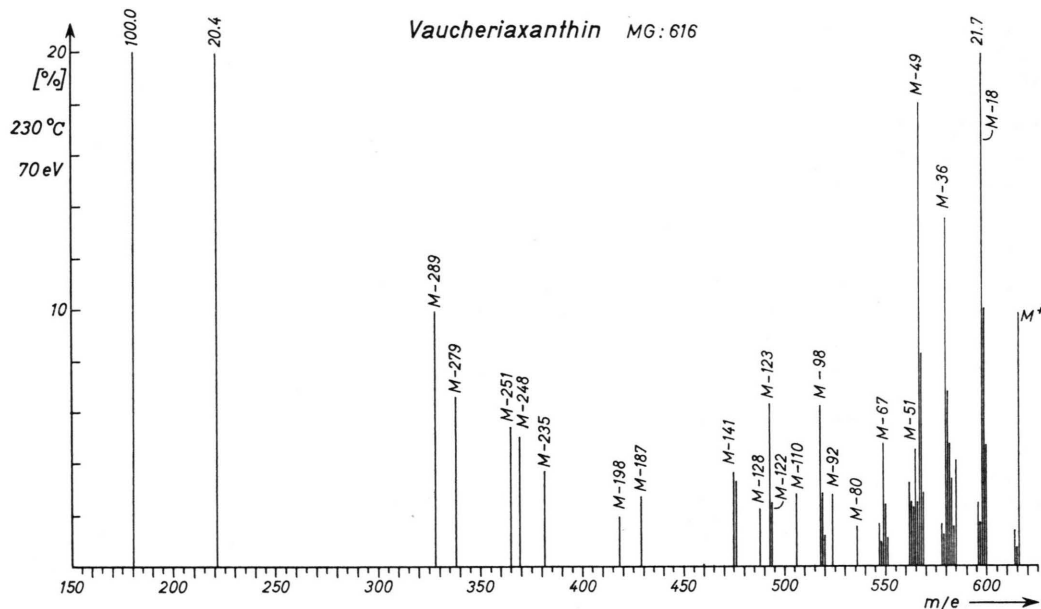


Fig. 3. Mass spectrum of vaucherixanthin.

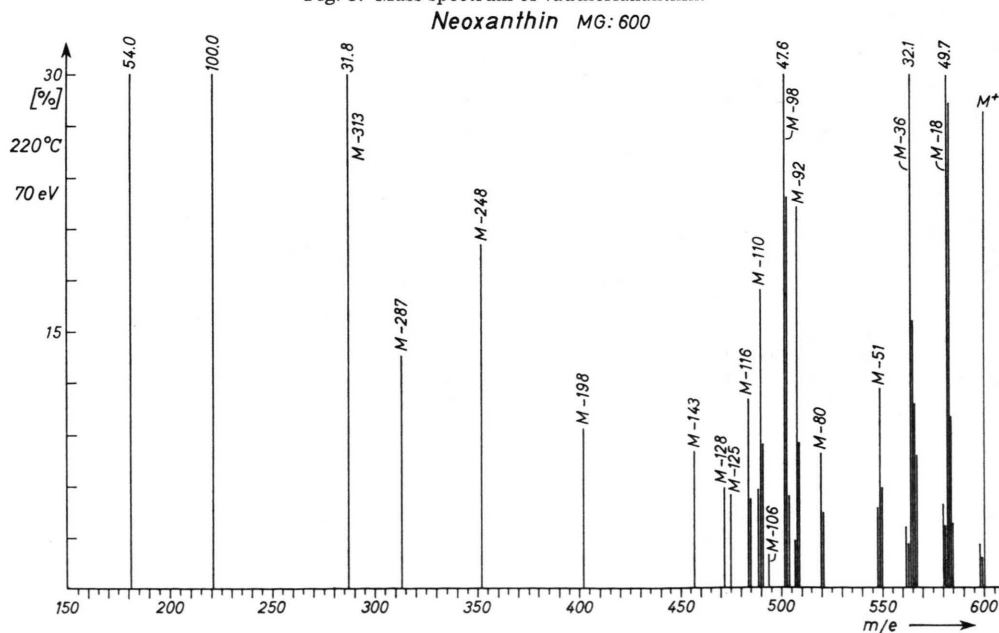


Fig. 4. Mass spectrum of neoxanthin.

MS of loroxanthin³, 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.*¹⁰ 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 eliminated¹¹. Therefore an other mechanism has been proposed, Fig. 5 B. If this scheme were correct, a substitution of the C₉ methyl should prevent the formation of the M-80 fragment and M-94

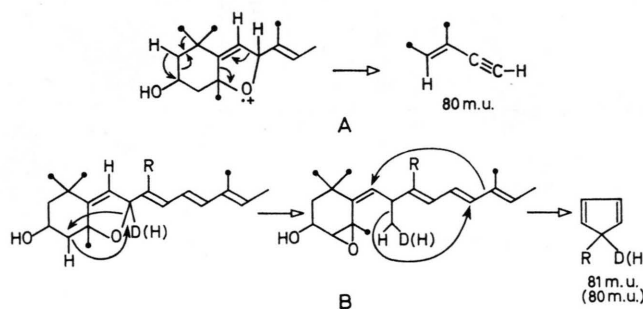


Fig. 5. Possible mechanisms of the formation of the M-80 fragment from xanthophyll-epoxides. A, according to Bonnett¹⁰. B, according to Budzikiewicz¹¹.

($R = \overset{\text{O}}{\parallel} C - H$)- M-96 ($R = CH_2OH$), M-110 ($R = CH_2OCH_3$), and M-138 ($R = CH_2 - O - \overset{\text{O}}{\parallel} C - CH_3$) should appear. This is not the case. *Vaucheriaxanthal*(diacetate) had M-80, M-93 = M-80-13 and M-98 besides a lot of additional M-80 derivatives, which are discussed below, but no line at M-94. *Vaucheriaxanthin* had M-80 and M-98 (Fig. 3), *vaucheriaxanthindimethylether* M-80 and M-112 = M-80-32 (Fig. 7), and *vaucheriaxanthintriacetate* M-80 and M-98 (M-138 here coincides with M-60-60-18). This has been the same with *loroxanthindioxide*, *loroxanthalepoxide* (no line at M-94) and *loroxanthinepoxidetriacetate* (no M-138 fragment) ².

Vaucheriaxanthin yielded a triacetate with mol. wt. 742 ($C_{46}H_{62}O_8$), Fig. 1 A, **2**, Fig. 6. Besides strong lines resulting from elimination of acetates (M-60, M-120) and acetate plus H_2O (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^{2,3}). But in the lower mass region a strong ion at m/e 410 (M-332) can

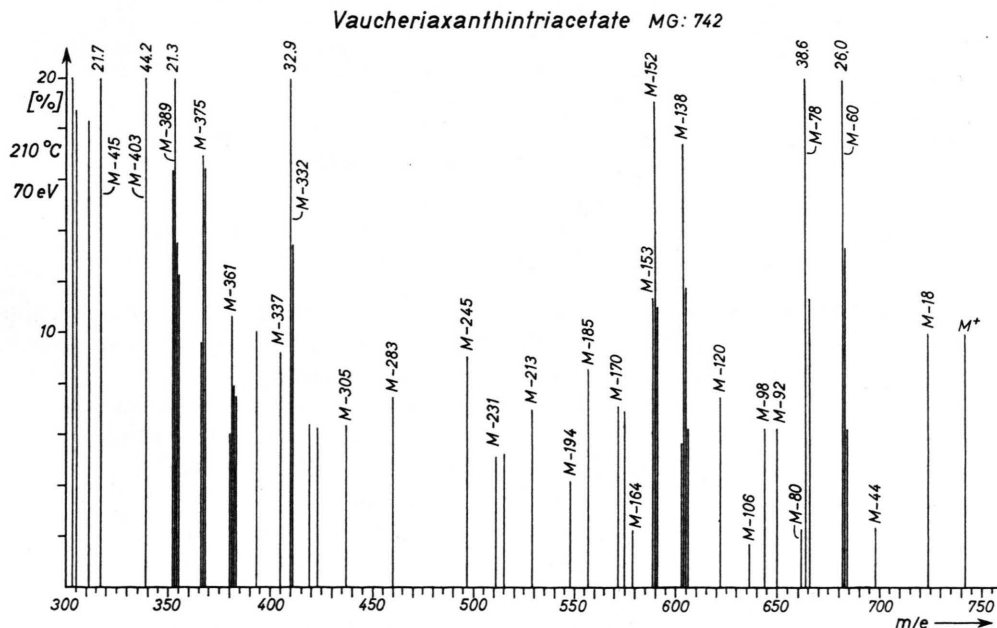


Fig. 6. Mass spectrum of vaucherixanthin-triacetate.

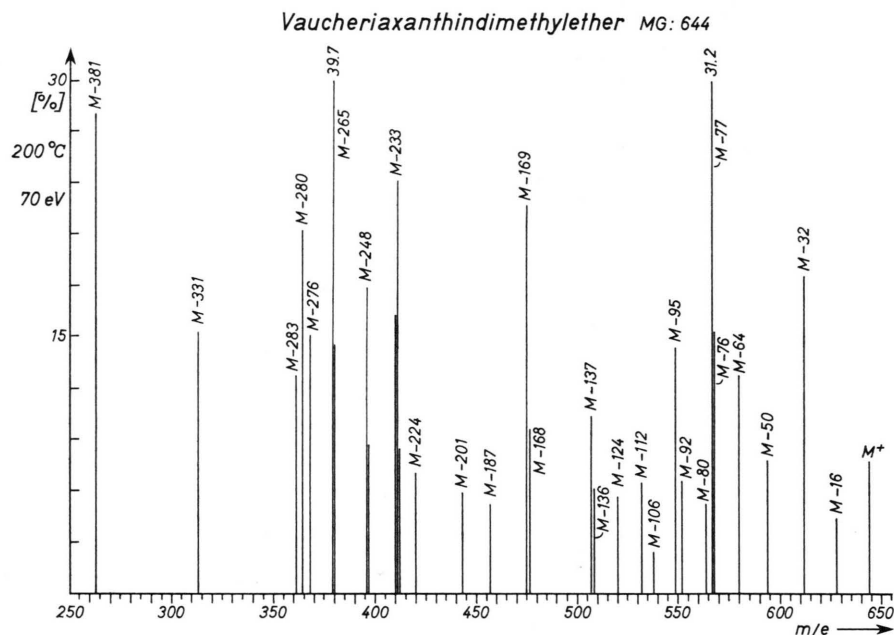


Fig. 7. Mass spectrum of vaucheriaxanthin-dimethylether.

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

| A | M — | 0 = M ⁺ | 34 (18–16) | 47 (18–16–13) | 60 | 78 |
|---|------|--------------------|------------|---------------|------|----------|
| | R.I. | 8.4 | 6.6 | 1.9 | 2.4 | 9.7 |
| A | M — | 80 | 92 | 93 | 98 | 106 |
| | R.I. | 4.6 | 6.0 | 11.2 | 19.1 | 2.3 |
| A | M — | 148 | 149 | 151 | 153 | 173 |
| | R.I. | 7.1 | 17 | 9 | 6.7 | 4.6 |
| A | M — | 185 | 277 | 295 | 317 | 330 |
| | R.I. | 5 | 6.5 | 5.4 | 8.4 | 7.3 |
| | | | | | | 343 |
| | | | | | | 32 |
| B | M — | 56 | 72 | 90 | 132 | 150 |
| | R.I. | 1 | 8.8 | 7.8 | 10 | 16.6 |
| B | M — | 192 | 206 | 210 | 224 | 242 |
| | R.I. | 4.4 | 5 | 6.2 | 3.3 | 2.6 |
| B | M — | 355 | 377 | 404 | 447 | 461 |
| | R.I. | 2.8 | 2.8 | 9.6 | 8.6 | 9 |
| | | | | | | 475 |
| | | | | | | 24.3 |
| | | | | | | 640 (BP) |
| | | | | | | 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.

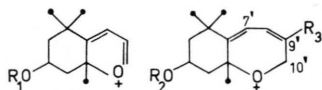
In contrast to the triacetate, the triacetate mono-silane (mol.wt. 814, $C_{49}H_{70}O_8Si$, Fig. 1 A, 3, Table I) had a line at M-164, which was accompanied by one at M-224 = M-164-60. M-92 and M-106 were completely absent, as was the mol-peak. Strong lines were at M-72 = M-56-16, M-132 = M-60-56-16, 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 origin is not yet clear.

Oxidation of the free pigment with *p*-chloroanil yielded a red coloured aldehyde, λ_{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, too². 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. Vaucherixanthaldiacetate had mol.wt. 698 ($C_{44}H_{58}O_7$). 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-185 = M-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 vaucherixanthindimethylester (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_2O (M-50) and methanol plus toluene (M-124). As in the free pigment and the vaucherixanthaldiacetate 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.



- 1 $R_1=R_2=H$; $R_3=CH_3$ m/e 181 m/e 221
- 2 $R_1=R_2=H$; $R_3=CH_2OH$ m/e 181 m/e 237
- 3 $R_1=R_2=CH_3CO$; $R_3=CH_2COOCH_3$ m/e 223 m/e 305
- 4 $R_1=R_2=CH_3CO$; $R_3=CHO$ m/e 223 m/e 277
- 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 vaucherixanthin and its derivatives.

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 221¹⁰⁻¹², which were formulated in Fig. 8. In the MS the line at m/e 221 always is more intense than that at m/e 181. *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 ($R_1:OH$, $R_2:CH_2OH$) m/e 181: m/e 237 is 3:1 (no line at m/e 221), in loroxanthaldiacetate 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 vaucherixanthin and its derivatives, too. Vaucherixanthin gave (Fig. 8, 2) 6:1, the triacetate (3) 8:1, vaucherixanthaldiacetate (4) 2.5:1, vaucherixanthin-dimethylether (5) 6:1 and the vaucherixanthintriacetate-monosilylether (3) 10:1. Similar results have been obtained from the MS of the allenic xanthophyll peridinin⁷, where the C_9 methyl is replaced by the carbonyl of a butenolide.

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

Material and Methods

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

Especially I wish to thank Dr. D. Wendisch (Bayer-AG, Leverkusen) for recording the 220 MHz PMR spectra. Dr. R. Maisch and Mr. R. Uhrich (Anorganic Chemistry Institute of the RWTH) again made the mass spectra, Mrs. H. Herrny (Organic Chem. Inst.) ran the infrared spectrum.

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