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Animal Carotenoids

6.* The Structures of Roserythrin and the Parent Nor-Carotenoid

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Structure *3*, a 2′-nor-astaxanthin diester, has been ascribed to a minor carotenoid isolated from the sea anemone *Actinia equina*. The structure is inferred mainly from that of roserythrin (*6*), obtained by careful alkali treatment of the natural ester. Several derivatives (*7* – *13*) of roserythrin have been prepared.

Roserythrin (*6*) was further prepared by partial synthesis from astacene (*5*). The properties of this partly synthetic roserythrin (*6*), including the electronic, IR and mass spectra, and of the derivatives *8* and *13* thereof, compared favourably with those obtained from roserythrin derived from the natural ester.

Only few true nor-carotenoids are known.

In part 3 of this series the carotenoids of the mauve variant of the sea anemone *Actinia equina* were examined.[1] The carotenoids encountered were all esterified. The major component, actinioerythrin (*1*, *ca.* 90 % of the total), was shown to be 2,2′-dinor-astaxanthin esterified with a variety of fatty acids in the $C_6 - C_{12}$ range. A second carotenoid (*ca.* 2 %) was found to be a diester (*2*) of astaxanthin. We now report on the structure of a third previously unidentified Ester X (*ca.* 8 %).

RESULTS AND DISCUSSION

Ester X [1] was adsorbed between the astaxanthin diester (*2*) and actinioerythrin (*1*) and exhibited absorption characteristics in visible light intermediate between those of *2* and *1*. Due to low abundance and oily contaminants Ester X could not be obtained in the pure crystalline state.

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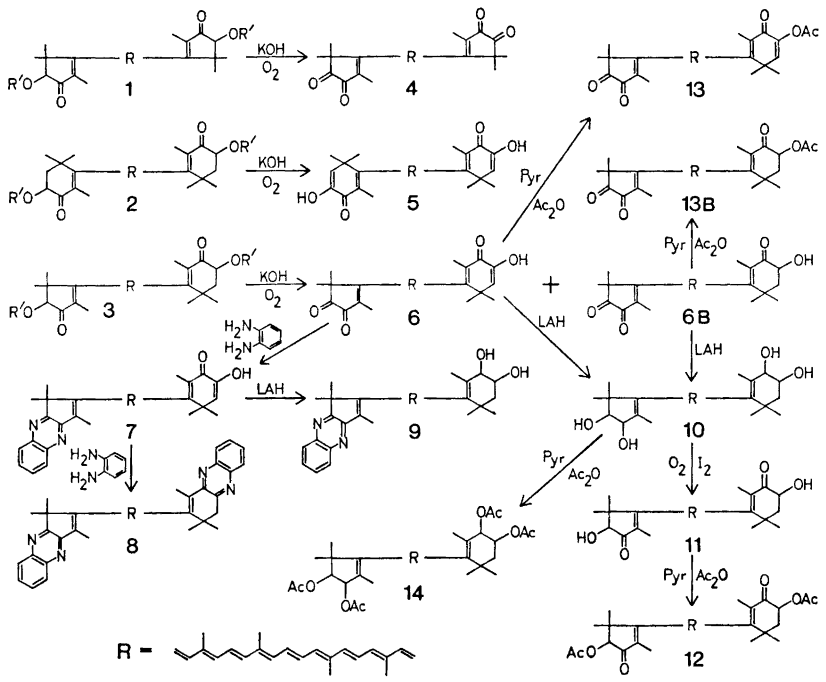
The structure *3* is inferred for Ester X from the product obtained on alkaline hydrolysis. On treatment of actinioerythrin (*1*) with weak alkali in the presence of oxygen the blue carotenoid violerythrin (*4*) is formed.[2,1] Similar treatment of *2* results in astacene (*5*),[3,1] and Ester X provided a product (*6*) with physical properties intermediate between those of *4* and *5*, Scheme 1. We have named this

SCHEME 1



compound roserythrin on account of its rose colour in solution and to indicate its relationship with violerythrin (*4*). Roserythrin (*6*) thus obtained was not free of non-carotenoid contaminants as judged by its melting point. Roserythrin (*6*) gave with *o*-phenylenediamine in a fast reaction a monoquinoxaline derivative (*7*) and then more slowly a bisquinoxaline derivative (*8*). The monoquinoxaline derivative (*7*) gave a reduction product (*9*) on treatment with lithium aluminium hydride, whereas the bisquinoxaline derivative (*8*) could not be reduced by this reagent.

Hydride reduction of roserythrin (*6*) gave a polar alcohol (*10*), which on allylic oxidation gave an ω,ω'-diketone. Structure *11* is assumed for the latter on the basis of its electronic spectrum and the formation of an acetate (*12*) on acetylation.

The absorption spectra in visible light of Ester X (*3*), 3,4,3′,4′-octahydroroserythrin (*10*) and the ω,ω'-diketone (*11*) suggested the presence of one five-membered ring of the type characteristic of actinioerythrin and one six-

membered ring of the astaxanthin type in *3*. This inference was supported by the electronic spectra of *4*, *5*, and *6*.

The IR spectrum of roserythrin (*6*) showed carbonyl absorption characteristic of violerythrin (*4*) in addition to weaker carbonyl bands attributable to both diosphenol (*cf.* astacene, *5*) and conjugated ketone (*cf.* astaxanthin) groupings. Like violerythrin (*4*), roserythrin (*6*) was unstable towards silylating reagents, and acetylation of *6* to give the enol acetate (*13*) was questionable. In particular, the partial mobility of the acetylated product on aluminium oxide paper was not expected for an enol acetate. The NMR spectrum obtained for roserythrin leant support to the structure *6*, and the mass spectrum of the bisquinoxaline derivative exhibited the molecular ion required for *8* (M = 722, M − 92, M − 106) [5] corresponding to $C_{51}H_{54}N_4$. However, the in-chain fragmentations expected by analogy with those of the equivalent astacene and violerythrin quinoxaline derivatives [1] were not as predicted.

Those of the above results not supporting structure *6* for roserythrin would be explicable on the basis of incomplete autoxidation of the six-membered ring α-ketol of Ester X under the conditions used to prepare roserythrin. This implies that our sample of roserythrin contained some 3′-dihydroroserythrin (*6b*) and that the acetate thereof contained some *13b*, Scheme 1. Such incomplete oxidation of flexixanthin by treatment with weak alkali [4] has been experienced. This hypothesis was supported by the mass spectrum of roserythrin acetate ultimately obtained: $M_{13b} = m/e$ 622 had intensity 56 % of that of $M_{13} = m/e$ 620, m/e 580 = $M_{13b} - 42$, m/e 578 = $M_{13} - 42$ or $M_{13b} - 44$. Astacene diacetate prepared for comparison exhibited M, M − 42, and M − 42 − 42 ions, whereas astaxanthin diacetate showed M, M − 44, and M − 44 − 44 ions.

When Holzel *et al.*[6] later effected a partial synthesis of violerythrin (*4*) by manganese dioxide treatment of astacene (*5*), the possibility of isolating roserythrin (*6*) as an intermediate was evident. It had been suggested that the biosynthesis of *1* involved a benzilic acid rearrangement,[1,7] and a similar mechanism was assumed for the *in vitro* oxidation of astacene. The intermediate *6* would thus be expected.

Heterogeneous oxidation of astacene (*5*) with manganese dioxide in acetone was carried out at room temperature. When the reaction was followed by paper and thin-layer chromatography an intermediate of polarity and visible absorption properties between those of *4* and *5* was observed. It proved possible to isolate this compound by quenching the reaction before completion by filtering off the oxidising agent. The intermediate, up to 20 % of the recovered carotenoid, could then be isolated by preparative thin-layer chromatography.

The intermediate thus isolated had absorption maximum in the visible region at 515 nm in acetone solution (Fig. 1), and this was in accordance with that expected for *6*, *cf.* data for *4* [1] and *5*.[4] Mass spectrometry revealed a molecular ion at m/e 578 (M, M − 92, M − 106) and base peak at m/e 44. A very intense ion at m/e 203 (42 %) has previously been described [8] as typical of the diosphenol end group found in *5*. The IR spectrum showed absorption compatible with structure *6*, see Fig. 2.

Acetylation of this product (*6*) with acetic anhydride in pyridine in the usual manner resulted in the formation of a monoacetate (*13*). This was

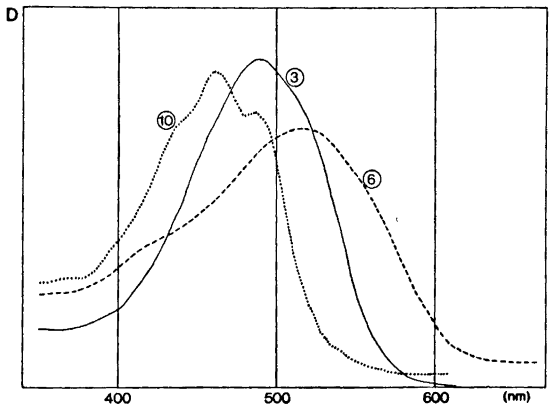


Fig. 1. Absorption spectra in visible light of ———— Ester X (2′-nor-astaxanthin diester, *3*), — — — roserythrin (*6*), and .... 3,4,3′,4′-octahydroroserythrin (*10*) in acetone.

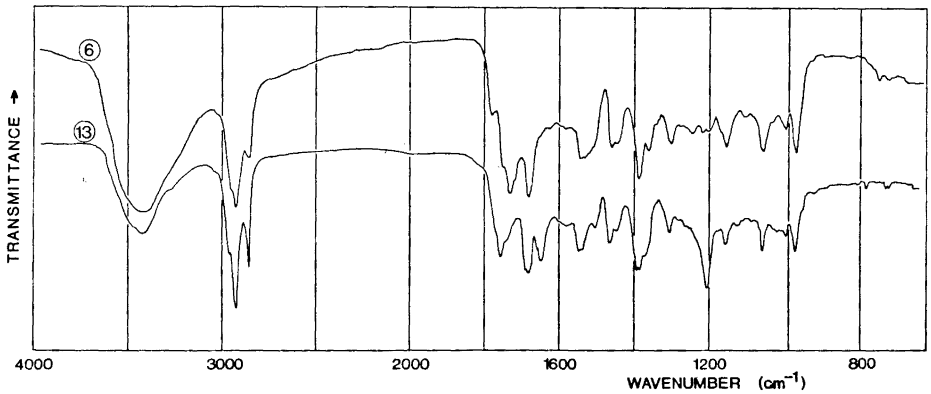


Fig. 2. IR spectra (KBr) of roserythrin (*6*) and roserythrin acetate (*13*) derived from astacene.

somewhat more polar than the parent compound on thin-layer chromatography although on kieselguhr paper the two compounds were inseparable. The mass spectrum showed a molecular weight of 620 (M, M − 92, M − 106, M − 158) and, as above, a relatively intense *m/e* 203 ion. The IR spectrum was very similar to that of the parent compound (see Fig. 2) showing only the changes expected on acetylation as judged by experience with astacene (*5*).[4]

Reduction of the intermediate (*6*) with lithium aluminium hydride in ether resulted in a hypsochromic shift in the visible region in accordance with that predicted for *10*. Acetylation of *10* gave an acetate different from violerythrol tetraacetate.

Finally the bisquinoxaline derivative (*8*) of the intermediate (*6*) was prepared as previously described for *4* and *5*.[1,3] This proved to have the expected molecular weight (M = 722) and showed a regular pattern of fragmentation of most of the in-chain bonds. Comparison of this spectrum (see Fig. 3) with those previously recorded [1] for the analogous quinoxaline deriva-

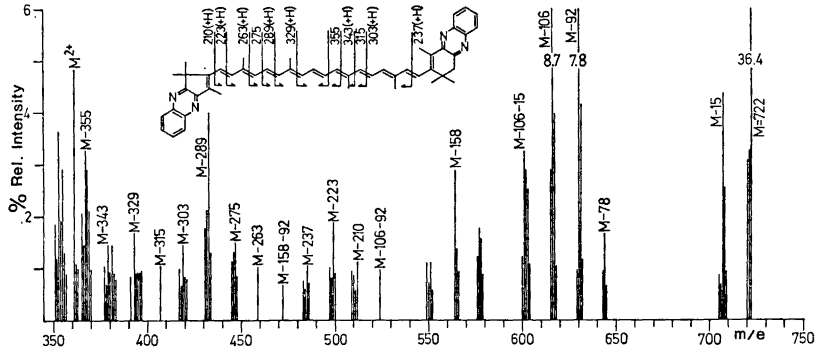


Fig. 3. Mass spectrum of roserythrin bisquinoxaline derivative (*8*) derived from astacene.

tives of *4* and *5* leaves no doubt as to the identification of the synthetic compound as *6*.

Examination of the data available for roserythrin and its derivatives together with co-chromatography of the parent compounds allowed the identification of roserythrin derived from Ester X with that obtained by oxidation of astacene (*5*).

It is therefore concluded that roserythrin has the structure *6* and that the compound described as Ester X has the structure *3*, a 2′-nor-astaxanthin diester. *3* is the third naturally occurring nor-carotenoid encountered; actinioerythrin (*1*) [1] and peridinin [9] have been described previously.

EXPERIMENTAL

Materials and methods. These were as stated elsewhere.[4,1] Thin layer plates were 0.5 mm layers of Merck Kieselgel G. Mass spectra were recorded on an AEI MS 902 machine using the direct inlet probe; ion source temperature *ca.* 200°C and electron bombardment energy 70 eV.

R_F-values and absorption maxima in visible light are compiled in Table 1.

Natural Ester X (*3*) and derivatives thereof

Biological material. The same source as described before was used.[1]

Isolation of the carotenoids. The procedure stated elsewhere was used.[1]

Ester X (*3*) was eluted from cellulose or kieselgel columns together with oily contaminants and *cis*-actinioerythrin (*1*). Judged from the yield of *6* below, the amount of *3* was roughly estimated as 8 % of the total carotenoid. Paper-chromatographically purified *3* (Fig. 1) gave on iodine catalyzed stereomutation a stereoisomeric set chromatographically different from that of *1*. In petroleum ether the *cis* peak of *3* was located at 376 nm, *cf.* 387 nm for *1*.

Roserythrin (*6*) was formed by alkaline hydrolysis of a mixture of Ester X (*3*) and *cis*-actinioerythrin (*1*), according to the procedure used for the transformation of *1* to *4*.[1] *6* was irreversibly adsorbed on alumina columns. Partial mobility (the major part remained irreversibly adsorbed) on aluminium oxide paper is ascribed to the presence of *6b*. Roserythrin was purified by chromatography on cellulose columns. Blue-black needles were obtained from acetone-petroleum ether; total yield *ca.* 4 mg. The best preparation melted at 200°C, but most samples melted below 82°C. The electronic spectrum is given in Fig. 1. The IR spectrum (KBr) had ν_{max}: 3400 (OH), 2920, 2850 (CH), (1780), (1750), 1730 (C=O in 3-position); 1690 (C=O in 4-position); 1660 (C=O in 4-position in *6b*); (*1620*, *1540* diosphenol); 1460, 1440 (CH_2); 1390, 1360 (methyl, *gem.* methyl); *1250*, 1220, 1200, 1155, *1060*, 1000 and 970 (*trans* disubst. double bonds) cm^{-1}. Italicized numbers indicate corresponding bands in the spectrum of astacene (*5*) attributed to its diosphenol groupings. The NMR spectrum ($CDCl_3$) exhibited methyl signals at τ 7.92, 7.98 (in-chain methyl) and 8.60 and 8.7 (*gem.* dimethyl). For astacene (*5*) are reported methyl signals at τ 7.90, 7.98 and 8.70,[10] and for violerythrin (*4*) at τ 7.93, 7.98 and 8.59.[6] The mass spectrum of *6* could not be obtained. On iodine catalyzed stereomutation of *6* no new zones were observed on kieselguhr paper.

6 in ether (abs.max. 504 nm) on treatment with KOH in methanol by the procedure used for violerythrin [1] gave spontaneously yellow hypophasic pigments (abs.max. 445 nm). Acidification of the hypophase caused a certain bathochromic shift, but reversal to *6* was not accomplished (single experiment). However, when a mixture of *4* and *6* was treated similarly both *4* and *6* were recovered after acidification of the alkaline hypophase.

Table 1. Absorption maxima in visible light and R_F-values of the carotenoids studied.

Carotenoid	Abs. max. nm in acetone			R_F-value system A			R_F-value system B	
				5 %	10 %	20 %	10 %	20 %
Astaxanthin diester (*2*)		480			0.65 [a]			
Ester X (*3*)		490			0.65			
Actinioerythrin (*1*)	(470)	496	529	0.11	0.52			
Astacene (*5*)		482			0.53			0.30
Roserythrin (*6*)		515			0.35	0.72		0.28
Violerythrin (*4*)		556			0.14	0.58		0.23
3,4,3′,4′-Tetraacetoxy-β-carotene	(427)	453	480		0.62		0.08	
3,4,3′,4′-Octahydroroserythrin tetraacetate (*14*)	(439)	461	489		0.66		0.13	
Violerythrol tetraacetate	(446)	470	500		0.71		0.20	
Roserythrin monoquinoxaline (*7*)		505			0.55			
Roserythrin bisquinoxaline (*8*)		506			0.72			
Astacene bisquinoxaline		486		0.58				
Violerythrin bisquinoxaline	(510)	530	565		0.34			
9 (reduced *7*)		498			0.15			
3,4,3′,4′-Octahydroroserythrin (*10*)	(439)	461	489			0.33		
3,3′-Tetrahydroroserythrin (*11*)		491				0.60 [b]		
12 (*11* acetate)		490			0.20			
Roserythrin acetate (*13*)		515			0.35			0.25
Astacene diacetate		482			0.41			0.25
"Red product" from MnO_2-ox.		485			0.26			0.30

System A: Schleicher & Schüll No. 287 kieselguhr paper. Developer acetone in petroleum ether.
System B: Kieselgel G. Above developer.

[a] 7 % and [b] 25 % acetone in petroleum ether.

Roserythrin monoacetate (13). *6* (1.0 mg, presumably mixed with *6b*) was submitted to acetylation conditions overnight; the pigment recovery was 80 %. The product could not be distinguished spectroscopically (visible light) or chromatographically (kieselguhr paper) from *6*. The IR spectrum (KBr) of the product corresponded to that of *6*, except for increased absorption around 1750 and 1200 cm^{-1}. For comparison astacene diacetate shows enol acetate absorption at 1768, 1200, and 1054 cm^{-1} (KBr). Dehydro-flexixanthin and its enol acetate exhibited identical R_F-values on kieselguhr paper and astacene and astacene diacetate have close R_F-values in the same system.[4]

Admixture of the product (*13*) with *13b* was suggested by partial mobility on alumina paper and the mass spectrum: *m/e* 622, 620, 580, 578, 105, 91.

Astaxanthin diacetate was prepared for comparison by acetylation of astaxanthin (4.1 mg) in the usual manner: *m/e* 680 (M), M − 44, M − 58, M − 60, M − 88 (= M − 44 − 44?), M − 92, M − 106, M − 158, 105 and 91. No M − 42 ion was recorded.

Astacene diacetate was similarly prepared: *m/e* 680 (M), M − 42, M − 42 − 42, M − 56 − 42, M − 58 − 42, M − 92 − 42, M − 92 − 42 − 42, M − 106 − 42, M − 106 − 42 − 42, M − 121, M − 121 − 42, M − 190, M − 194, M − 207, M − 207 − 42, M − 247, M − 247 − 42, M − 259, M − 259 − 42, M − 273, M − 273 − 42, 245, 203, 165, 151, 119, 105, 91, 83, 55, 43.

3,4,3′,4′-Octahydroroserythrin (10). *6* (0.1 mg) was reduced with $LiAlH_4$ in ether; pigment recovery was 50 %. The product consisted of *10* exclusively; for properties see Fig. 1 and Table 1.

3,3′-Tetrahydroroserythrin (11). *10* (30 μg) was oxidized by air in the presence of iodine.[1] The final product was *11*, less strongly adsorbed than *10*, and provided the acetate (*12*) on acetylation.

Reaction of 6 with o-phenylenediamine. *6* (0.2 mg) in acetic acid (1 ml) was treated with *o*-phenylenediamine (10 mg) at 100°C for 4 h. The pigment recovery was 50 %. The reaction mixture contained *7* (70 % of total) and *8* (30 %). Periodic chromatographic analysis during the reaction revealed the transformation of *7* to *8*.

Roserythrin monoquinoxaline derivative (7). *7*, reduced with $LiAlH_4$ in ether, gave *9*.

Roserythrin bisquinoxaline derivative (8). The mass spectrum of *8* had prominent peaks in the upper mass region at *m/e* 722 ($M = C_{51}H_{54}N_4$), M − 15, M − 78, M − 92, M − 106, M − 120, M − 145, M − 171, M − 173, M − 185, M − 191, M − 213, M − 224, M − 229, and M − 239.

8 could not be reduced with $LiAlH_4$ in ether.

Partial synthesis of roserythrin (*6*) from astacene (*5*)

Oxidation of astacene. The method of Holzel *et al.*[6] was adopted. A typical experiment was as follows: Astacene (*5*, 4.18 mg) was dissolved in acetone (5 ml) and manganese dioxide (50 mg) added. The reaction mixture was stirred in the dark and under nitrogen cover for 2 h at room temperature. Small samples were removed periodically to check the reaction by thin-layer and paper chromatography. The reaction was stopped by addition of acetone (50 ml) and immediate filtration. The manganese dioxide was further washed with acetone (*ca.* 200 ml) and the filtrates combined. The recovered pigment represented 47 % of the starting material (estimated spectroscopically using $\varepsilon = 1690$ for astacene [10]).

The recovered pigment was chromatographed on thin-layer plates using 20 % acetone in petroleum ether as solvent. Four zones were produced: astacene (*5*, 57 %), roserythrin (*6*, 17 %), a red product (11 %) and violerythrin (*4*, 14 %). Astacene (*5*) and violerythrin (*4*) were identified by co-chromatography with genuine pigments.

The red product was never obtained free from astacene (*5*), but apparently had a very similar electronic spectrum. The mass spectrum exhibited peaks at *m/e* 576 (M), 574 (M − 2?), 203 (astacene end group). $LiAlH_4$ reduction in ether resulted in a hypsochromic shift from *ca.* 485 nm to *ca.* 450 nm (acetone).

Roserythrin (6). The electronic spectrum is given in Fig. 1. The IR spectrum had ν_{max}: 3400 (OH); 2920, 2850 (CH); 1730 (C=O in 3-position); 1680 (C=O in 4-position); 1620, 1540 (diosphenol); 1460, 1440 (CH_2); 1390, 1360 (methyl, *gem.* dimethyl); 1250, 1220, 1200, 1155, 1060, 1000, and 970 (*trans* disubst. double bonds) cm^{-1}. The mass spectrum had *m/e* 578 (M), M − 92, M − 106, 412, 394, 383, 203 (42 %), 105, 91, 44 (100 %).

Roserythrin acetate (13). Acetylation of roserythrin (*6*) was carried out in the normal manner. The IR spectrum had ν_{max} 3400; 2920, 2850 (CH); 1755 (acetate) 1730 (3-keto);

1680 (4-keto), 1650 (4′-keto), 1550; 1460, 1440 (CH_2); 1390, 1360 (methyl, *gem.* dimethyl); 1210 (enol acetate); 1150, 1060, 1000; 970 (*trans* disubst. double bonds) cm^{-1}. The mass spectrum had *m/e* 620 (M), M − 16, M − 42, M − 58, M − 92, M − 106, M − 92 − 42, M − 106 − 42, M − 158, 203, 105, 91, 43.

3,4,3′,4′-Octahydroroserythrin tetraacetate (14). Roserythrin (*6*) was reduced with excess $LiAlH_4$ in ether. The product (*10*) was acetylated in the usual manner to furnish the peracetate (*14*).

Roserythrin bisquinoxaline derivative (8). *8* was prepared from *6* by the procedure given above. The mass spectrum with assignments is given in Fig. 3.

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