Animal Carotenoids. 9*. Trikentriorhodin

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In his thesis Smith [1] described the quantitative distribution pattern of carotenoids in the marine sponge *Trikentrion helium* Dickinson (family Cyamonidae) and a partial characterization of the individual carotenoids. The structure of a major carotenoid P474, now called trikentriorhodin, occurring both in esterified and unesterified form [1] has been further studied in our laboratory.

The work was complicated by the presence of much lipid contaminants. Esterified trikentriorhodin (26 % of total carotenoids) on saponification gave free trikentriorhodin (*1*), identical with naturally occurring trikentriorhodin (comprising 21 % of total carotenoids). The structural assignment is based on the following evidence; the position of the hydroxy group in the cyclopentane end group being tentative. Formally trikentriorhodin (*1*) is 3-hydroxy-$\varkappa,\chi$-caroten-6,8-dione. Trikentriorhodin (*1*) exhibited a round-shaped absorption spectrum in all solvents with λ_{max} 518 mn in CS_2; 497 nm in pyridine; (468), 488 and (520) nm in petroleum ether, 478 nm in acetone and 475 nm in diethyl ether, bathochromically displaced relative to astaxanthin [2] and hypsochromically shifted relative to rhodoxanthin,[3] caused by a so far unknown chromophore.

The molecular formula $C_{40}H_{52}O_3$ (*m/e* 580.3908, calc. 580.3917) was established by high precision mass spectrometry.

Fragment ions at M − 92 and M − 106 [4] confirm this assignment. The occurrence of trikentriorhodin as an ester and a prominent M − 18 ion in the mass spectrum of the hydrolyzed pigment indicated the presence of hydroxyl. Trikentriorhodin (*1*) was destroyed under standard conditions for silylation [5] or acetylation,[5] although a monoacetate *1b* (*m/e* 622 = M, M − 60) with unchanged electronic spectrum was obtained in low yield. Attempted methylation of allylic hydroxyl with HCl-MeOH [6] gave 60 % recovery of *1* and no methyl ether, under conditions where lutein gave the monomethyl ether [7] in high yield. Treatment with NaH/MeI [8,9] caused complete decomposition and gave no methyl ether. Attempted allylic oxidation with I_2/O_2 [10] or DDQ [11] again caused decomposition and provided no oxidation products. Trikentriorhodin (*1*) thus appeared to contain a non-allylic hydroxy group, the localization of which was further indicated from the mass spectrum with diagnostically useful fragment ions at M − 127 and *m/e* 127 (*a*, 22 % of base peak) and base peak *m/e* 109.1013 (calc. 109.1017 for $C_8H_{13} = b$, consistent with $b = a - 18$), compatible with a hydroxylated $\varkappa$ [12] end group,[13] Scheme 1.

Careful $LiAlH_4$-reduction gave more polar products with hypsochromically displaced electronic spectra, see below, demonstrating the presence of conjugated carbonyl. IR-absorption at 1610, 1568 cm^{-1} of trikentriorhodin compatible with enolized α- or β-diketone,[14] suggested an end group similar to that recently established by Khare *et al.* in mytiloxanthin (*2*).[15] Support was derived from an M − 155 peak [15] in the mass spectrum of *1*, attributed to α-cleavage, and ^1H NMR signals ($CDCl_3$) at δ 5.83 s (1 H) [15] and methyl singlets at δ 0.85, 1.20 and 1.34 [15,16] compatible with the $\varkappa$ end

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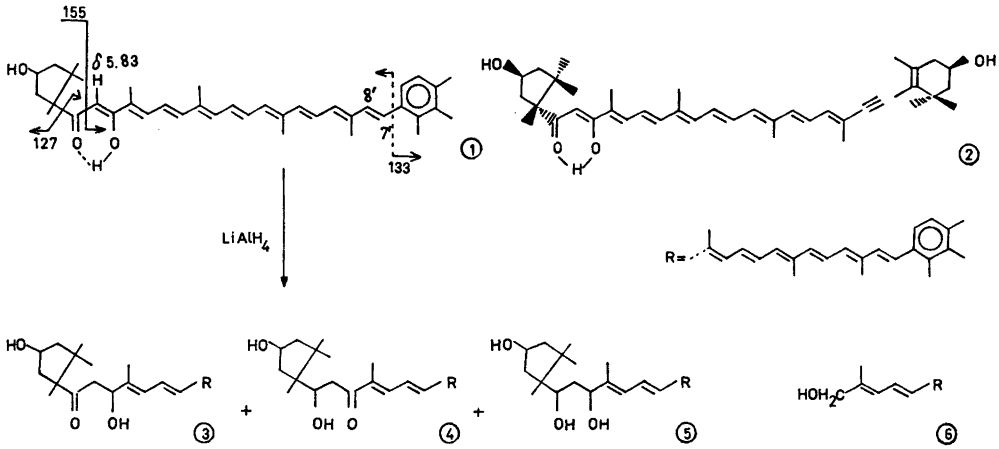

Scheme 1.

group. Weak acidic properties of the enolized β-diketone was consistent with irreversible adsorption of trikentriorhodin (*1*) on alumina, although less evident from partition tests without and in the presence of alkali.

Having now accounted for all oxygen functions, consideration of the chromophore and the remaining double bond equivalences, require that the second end group should be a trimethylphenyl end group. Confirmation was obtained from a broad, unresolved ^{1}H NMR-signal at δ 1.8–2.3 compatible with in-chain methyl and aromatic methyl.[4] Absence of M – 133 and *m/e* ions in the mass spectrum of *1* is ascribed to other preferred cleavages. Preference for 1,2,3-trimethyl substitution follows from the electronic spectrum of trikentriorhodin (*1*) and derivatives to be discussed next.

Micro scale $LiAlH_4$-reduction of *1* gave products with spectral properties compatible with the conjugated ketone *4* [λ_{max} in ether 456, (482) nm], the unconjugated ketone *3* (*m/e* 582 = M, M – 18); λ_{max} in ether 335, 351, 441, 468 nm), and the allylic alcohol *5* (λ_{max} in ether 335, 350, 441 and 465 nm; *m/e* 584 = M, M – 18, M – 18 – 18, M – 106 – 18, M – 158).

Direct comparison of the electronic spectra in diethyl ether of the triol *5* (λ_{max} 441, 468 nm) with that of renierol (*6*, λ_{max} 439, 465 nm), prepared by $LiAlH_4$-reduction of renieral, require 1,2,3- rather than 1,2,5-trimethyl substitution of the aryl ring; *cf.* Ref. 18. Consideration of the absorption maximum of mytiloxanthin (*2*) [15] at lower wavelength than that of trikentriorhodin (*1*) leads to the same conclusion. Evidence obtained by Smith [1] suggests the presence of β-isorenieratene with a 1,2,3-trimethylphenyl end group in *T. helium*.

Experimental. Methods were as commonly employed in this laboratory.[19,5] The isolation involved extraction with acetone, removal of colourless contaminants by deep-freezing, followed by filtration of the concentrated acetone extract, repeated TLC (silica gel G, 1 mm, 5 % acetone in petroleum ether), standard saponification of individual fractions, again followed by repeated TLC of saponified pigments. The carotenoid composition of *T. helium* studied here corresponded to that found by Smith;[1] available *ca.* 7 mg, purified *1*.

R_F-values on Schleicher and Schüll No. 287 kieselguhr paper in acetone-petroleum ether (APE) were: β-carotene 1.00 (0 % APE), *1* 0.29 (5 % APE), *1*-esters 0.27, 0.31 (0 % APE). Relative polarity on silica gel G plates (20 % APE) were *7* < *1* < *3* < *4* < *5*.

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