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Bacterial Carotenoids

XXXIII.* Carotenoids of Thiorhodaceae 9** The Structures of the Carotenoids of the Rhodopinal Series

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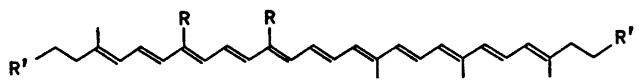
Mass spectrometry of the natural carotenoids and various derivatives has, in combination with previous results, permitted structural assignments of lycopenal (*3*), rhodopinal (*4*), rhodopinol (*6*), and 3,4,3′,4′-tetrahydrospirilloxanthinal (*9*). A previously assumed lycopenal presumably is 1,2-dihydro-1-methoxy-lycopen-20-al (*10*).

A plausible biosynthetic route to these carotenoids is briefly discussed.

In addition to lycopene and rhodopin with well established structures, the cross-conjugated aldehydes rhodopinal, lycopenal, 3,4,3′,4′-tetrahydrospirilloxanthinal, and the corresponding allylic alcohols rhodopinol [1] and lycopenol [2] are regarded as members of the rhodopinal series, carotenoids characteristic of various photosynthetic purple sulphur bacteria. These compounds, in which an in-chain methyl group is formally oxidized, have been assigned gross structures,[1] exemplified by *1* for the key member of the series, rhodopinal. Previous chemical and spectroscopic evidence revealed that the cross-conjugated aldehyde group in *1* was located in 19, 20, 19′, or 20′ position. Some preference for 20 or 20′ position was derived from the intense *cis*-peak or rhodopinol (*2*)[1] ascribed to a *cis*-bond adjacent to the hetero substituent in near-to-central position.[1,3] However, the data available could not decide the position of the hydroxylated end group relative to the aldehyde group.



R′ = >= and HO >—

1. R = CH_3 and CHO

2. R = CH_3 and CH_2OH

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RESULTS AND DISCUSSION

Previous attempts to obtain mass spectra of these carotenoids were largely unsuccessful.[1] Schwieter *et al.*[4] have recently concluded that mass spectra of carotenoids are partly produced by pyrolysis products. The present carotenoids suffered more thermal decomposition than experienced in previous work on carotenoids with a normal methyl substituted polyene chain. However, informative mass spectra were obtained for lycopenal (*3*), rhodopinal (*4*), LAD-reduced rhodopinal (*5*), rhodopinol (*6*), rhodopinol acetate (*7*), rhodopinol D_3-acetate (*8*), and 3,4,3′,4′-tetrahydrospirilloxanthinal (*9*).

The mass spectra confirmed the molecular weights of the compounds. The identification of the molecular ions was supported by M − 92 (P), M − 106 (Q), and M − 158 (T) peaks in most cases [5–7] (Table 1). In addition to the common P, Q, and T peaks P′, Q′, and T′ peaks with appropriate mass shift caused by the extra substituent on the polyene chain [8,9] were abundant (Table 1) and confirmed the location of the allylic oxygen substituent at the 19, 20, 19′, or 20′ positions.[8]

Preference for substitution in the 20-position follows from ions ascribed to in-chain cleavages. Previously in-chain cleavages have mainly been encountered in cyclic carotenoids.[7,10] However, oxygenated substituents on the polyene chain may promote such fragmentation, and several ions compatible with in-chain fragmentations were observed.[11] Accurate mass determinations were subsequently attempted on a number of significant ions; see the respective mass spectra. The ions accurately determined are ringed on the spectra and similarly marked on the structures. Values are given in the Experimental part. The results for rhodopinol strongly support the formulation 1-hydroxy-1,2-dihydro-lycopen-20-ol (*6*); see Fig. 1. The observed in-chain fragmentations of rhodopinal are also in agreement with the 20-al structure (*4*), although in this case 20′- or 19′-substitution could not be disregarded from mass spectrometric data alone. However, since the exact chemical relationship between rhodopinol (*6*), rhodopinal, and lycopenal have been demonstrated [1] (Fig. 1), the structures of rhodopinal (*4*) and lycopenal (*3*) are given. Since the chromophore of 3,4,3′,4′-tetrahydrospirilloxanthinal is identical with those of the other cross-conjugated aldehydes (*3*, *4*),[1] 20-al substitution is concluded for the former compound (*9*) too, Fig. 1.

The mass spectra of *3* − *9* further confirmed the previous [1] end group assignments. The *m/e* 69 ion was intense in the spectra of *3* − *6* (base peak) and *7* (59 %), *8* (39 %), and *10* (28 %). This observation and the occurrence of M − 69 ions for *3* and *4* confirmed the presence of lycopene-type (isopropylidene) end groups. Rhodopinal (*4*) and rhodopinol (*6*) showed intense M − 18 ions which confirmed the presence of hydroxy groups. The M − 60 and M − 63 ions in the spectra of the acetates *7* and *8* are due to loss of acetic acid. In the dimethoxy compound *10* loss of methanol gives rise to M − 32 and M − 32 − 32 ions. Similar losses were observed from other prominent ions. Furthermore the base peak at *m/e* 73 confirmed the presence of tertiary methoxy groups in 1,1′-position in *10*.[7]

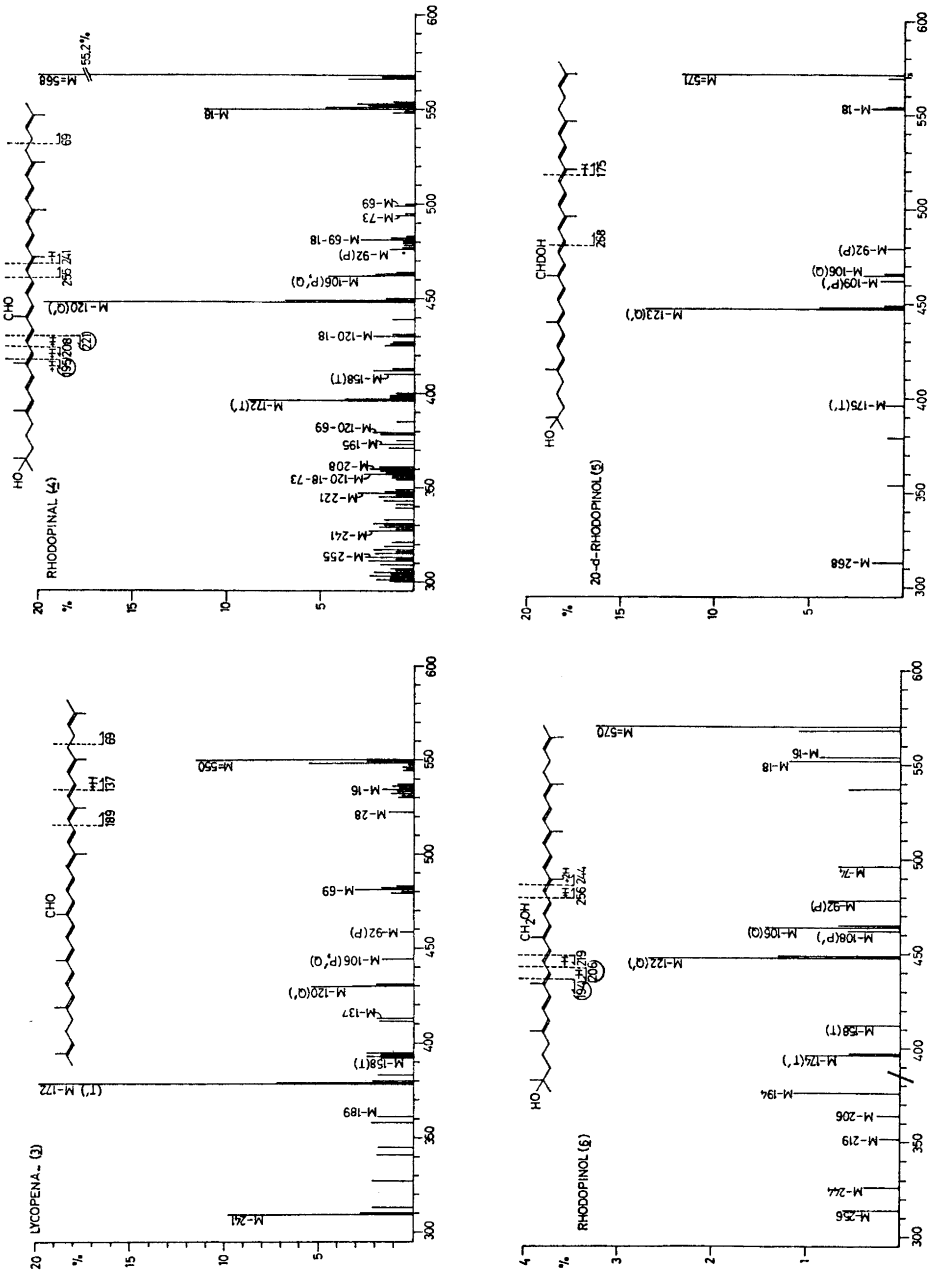
A carotenoid previously isolated from a *Lamprocystis* sp. strain 3012 and identified as lycopenal [2] was shown by mass spectrometry to be the mono-

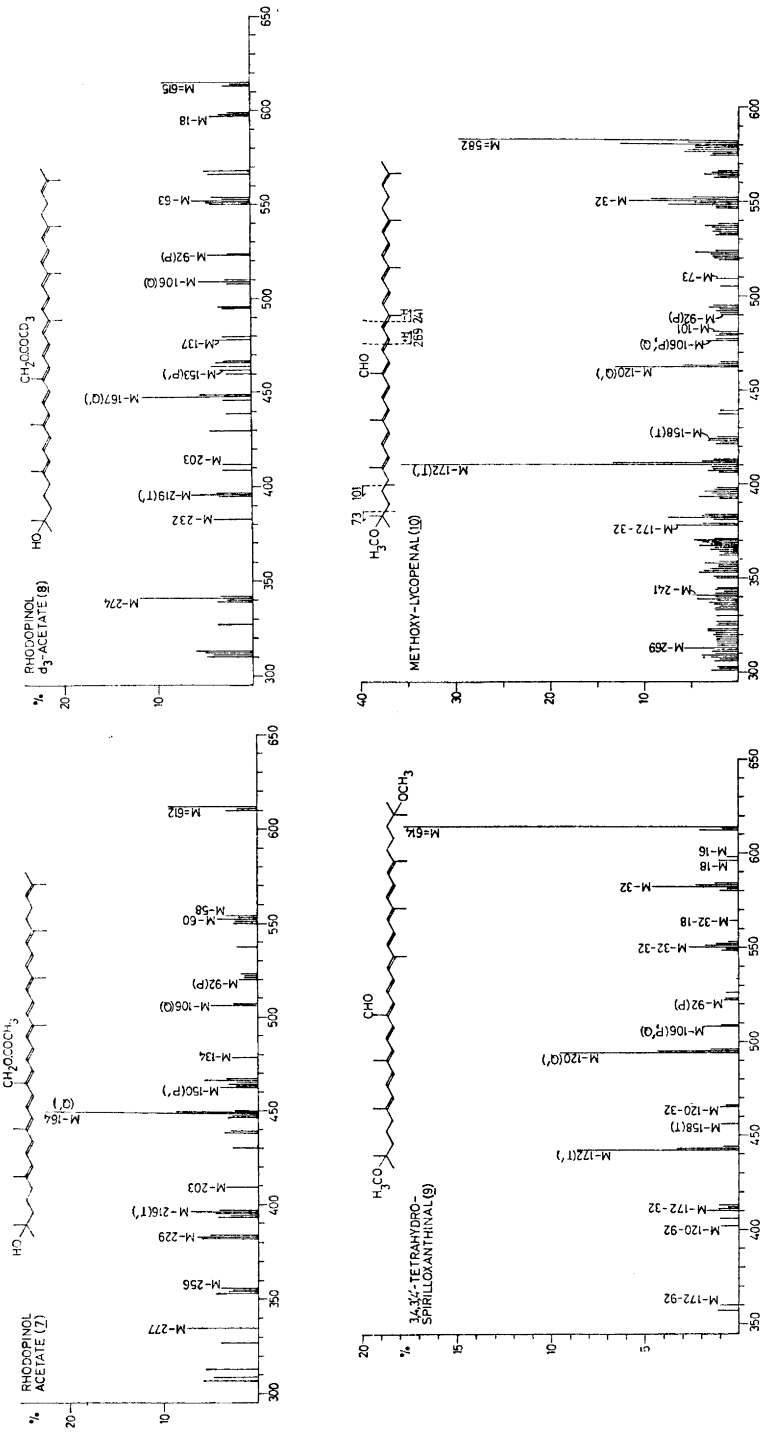
Table 1. Ions caused by excision of part of the polyene chain in various carotenoids.

Carotenoid	In-chain substituents		Ions observed (*m/e*)					
	A[a]	B[a]	P	P′	Q	Q′	T	T′
Normal[b]	CH_3	$3\times CH_3$	M−92		M−106		M−158	
3	CHO		M−92	*M−106*	*M−106*	M−120	M−158	*M−172*
4	CHO		M−92	*M−106*	*M−106*	*M−120*	M−158	M−172
9	CHO		M−92	M−106	M−106	M−120	M−158	M−172
10	CHO		M−92	M−106	M−106	*M−120*	M−158	*M−172*
6	CH_2OH		*M−92*	*M−108*	*M−106*	*M−122*	M−158	M−174
5	CHDOH		*M−92*	*M−109*	*M−106*	*M−123*	M−158	M−175
7	CH_2OCOCH_3		M−92	M−150	M−106	M−164		M−216
8	CH_2OCOCD_3		M−92	M−153	M−106	M−167		M−219

[a] Neutral fragments lost in formation of P, Q, T ions do not contain A. Fragments lost in formation of P′, Q′, T′ ions always contain A. B is absent from the neutral fragment lost in formation of P, P′ ions and present once in fragments lost in formation of Q, Q′, T, T′ ions.

[b] No oxygenated in-chain substituents; lateral methyl groups only. Where accurate mass measurements have been carried out numbers are given in italics.


RHODOPINAL (4)
20-d-RHODOPINOL (2)
LYCOPENAL (3)
RHODOPINOL (1)


RHODOPINOL d3-ACETATE (8)
METHOXY-LYCOPENAL (10)
RHODOPINOL ACETATE (7)
3,4,3',4'-TETRAHYDRO-SPIRILLOXANTHINAL (9)

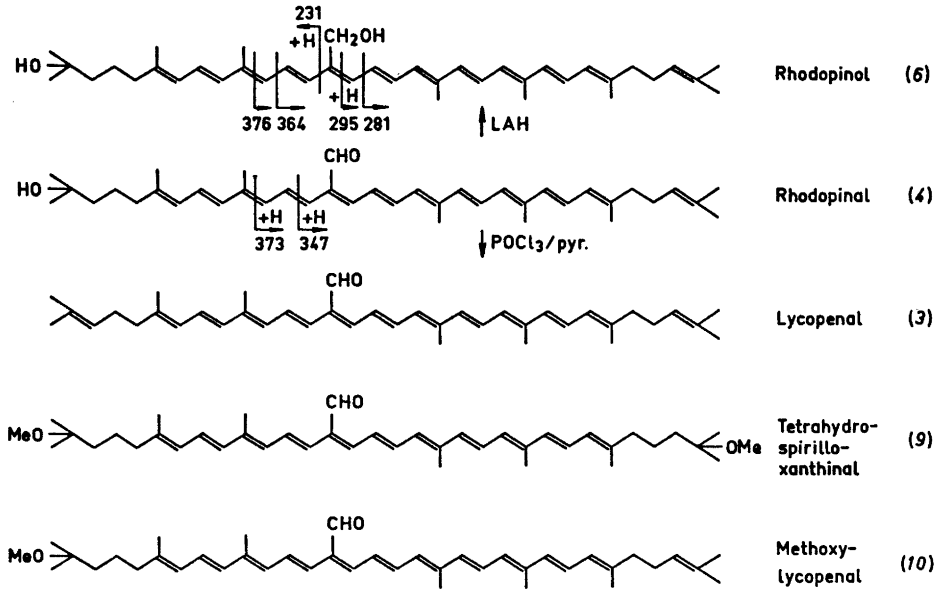


Fig. 1. Structures of the carotenoids of the rhodopinal series. The formulae are drawn with all-*trans* polyene chain, but all compounds are assumed to possess *cis*-configurations at the 13-double or 12-single bond.[1,3] Only fragmentations based on exact mass measurements are indicated.

methoxy derivative with one methoxylated end group of 3,4,3′4′-tetrahydrospirilloxanthin-type (strong *m/e* 73 ion) and one lycopene-type end group (strong *m/e* 69 ion). In this case the results do not permit a definite differentiation between 20- and 20′-al substitution, although tentatively assigned in-chain fragmentations and analogy with the compounds above favour 20-substitution in this case too; see Fig. 1. In view of the present findings the presumed lycopenol in *Lamprocystis* strain 3012[2] may be a methoxylated derivative too.

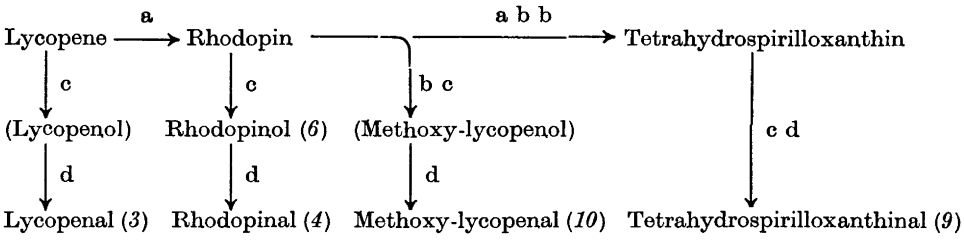


Fig. 2. Postulated biosynthetic pathway for carotenoids of the rhodopinal series.

The biosynthesis of the rhodopinal series of carotenoids is likely to occur *via* lycopene, and the formation of the various members (Fig. 2) may proceed by the following four steps: a) Hydration of an isopropylidene end group with formation of a tertiary alcohol. b) Methylation of the latter. Both these reactions are thought to operate also in the biosynthesis of other carotenoids in Thiorhodaceae,[12] and the reactions leading to the unique cross-conjugated aldehydes are further: c) Oxygenation of the central methyl group closest to any terminal oxygen substituent, and d) Oxidation of the primary allylic hydroxy group to give the aldehydes (*3*, *4*, *10*, *9*). Some variation in the order of these steps may also be considered.

EXPERIMENTAL

Rhodopinal (*4*) and rhodopinol (*6*) were obtained from a *Thiocystis* sp.[1] and lycopenal (*3*) from the same *Thiocystis* sp. as well as *Chromatium warmingii* strain Melbourne.[1] 3,4,3′,4′-Tetrahydrospirilloxanthinal (*9*) had been isolated from a *Thiococcus* sp.[13,1] 1-Methoxy-1,2-dihydrolycopenal (*10*) was obtained from a *Lamprocystis* sp. strain 3012.[2]

5 was prepared by LAD-reduction of rhodopinal (*4*) in dry ether. *7* and *8* were prepared by acetylation of rhodopinol (*6*) with acetic anhydride and hexadeutero acetic anhydride, respectively, in dry pyridine.

All compounds studied were re-chromatographed on neutral alumina columns (activity grade 2) prior to recording the mass spectra on an MS 902 instrument. A small amount of a concentrated acetone solution was evaporated to dryness in the direct inlet probe before introduction. In each case optimum condition was sought by varying the ion source temperature and electron voltage of the bombarding electrons. When the sample was kept in the instrument for some minutes prior to recording, the resultant spectrum was not carotenoid in nature. In general the reproducibility of the spectra was lower than usual.

Spectra of the upper mass region of each compound studied, given in the figures, were obtained at 70 eV (except 20 eV for *3* and *10*) and at the minimum ion source temperature (180–230°C) required to obtain volatilization of the sample.

Accurate mass measurements are cited below. Attempts were made to determine the constitution of a number of other ions, but continuous variation, both in the intensity of these and the species contributing to particular *m/e*-values made further peak matching unsuccessful. Normally such unsuccessful attempts were due to the presence of highly oxygenated ions of transitory nature.

Lycopen-20-al (*3*). M−106 (M−C_8H_{10}), measured *m/e* 444.338, calc. 444.339 for $C_{32}H_{44}O$; M−106 (M−C_7H_6O), measured *m/e* 444.378, calc. 444.376 for $C_{33}H_{48}$; M−172 (M−$C_{12}H_{12}O$), measured *m/e* 378.327, calc. 378.329 for $C_{28}H_{42}$.

Rhodopin-20-al (*4*). M−106 (M−C_8H_{10}), measured *m/e* 462.350, calc. 462.350 for $C_{32}H_{46}O_2$; M−106 (M−C_7H_6O), measured *m/e* 462.386, calc. 462.386 for $C_{33}H_{50}O$; M−120 (M−C_8H_8O), measured *m/e* 448.370, calc. 448.370 for $C_{32}H_{48}O$; M−195 (M−$C_{13}H_{23}O$), measured *m/e* 373.252, calc. 373.253 for $C_{27}H_{33}O$; M−221 (M−$C_{15}H_{25}O$), measured *m/e* 347.236, calc. 347.237 for $C_{25}H_{31}O$.

20-D-Rhodopin-20-ol. (*5*). M−106 (M−C_8H_{10}), measured *m/e* 465.371, calc. 465.372 for $C_{32}H_{47}DO_2$; M−109 (M−C_7H_7DO), measured *m/e* 462.386, calc. 462.386 for $C_{33}H_{50}O$; M−123 (M−C_8H_9DO), measured *m/e* 448.370, calc. 448.371 for $C_{32}H_{48}O$.

Rhodopin-20-ol (*6*). M−92. (M−C_7H_8), measured *m/e* 478.380, calc. 478.381 for $C_{33}H_{50}O_2$; M−106 (M−C_8H_{10}), measured *m/e* 464.365, calc. 464.365 for $C_{32}H_{48}O_2$; M−108 (M−C_7H_8O), measured *m/e* 462.386, calc. 462.386 for $C_{33}H_{50}O$; M−122 (M−$C_8H_{10}O$), measured *m/e* 448.370, calc. 448.371 for $C_{32}H_{48}O$; M−174 (M−$C_{12}H_{14}O$), measured *m/e* 396.340, calc. 396.339 for $C_{28}H_{44}O$; M−194 (M−$C_{13}H_{22}O$), measured *m/e* 376.279, calc. 376.277 for $C_{27}H_{36}O$; M−206 (M−$C_{14}H_{22}O$), measured *m/e* 364.279, calc. 364.277 for $C_{26}H_{36}O$; M−275 (M−$C_{18}H_{27}O_2$), measured *m/e* 295.243, calc. 295.243 for $C_{22}H_{31}$; M−289 (M−$C_{19}H_{29}O_2$), measured *m/e* 281.226, calc. 281.227 for $C_{21}H_{29}$; M−339 (M−$C_{24}H_{35}O$), measured *m/e* 231.176, calc. 231.175 for $C_{16}H_{23}O$.

1-Methoxy-1,2-dihydrolycopen-20-al (*10*). M, measured *m/e* 582.442, calc. 582.444 for $C_{41}H_{58}O_2$; M−32 (M−CH_4O), measured *m/e* 550.414, calc. 550.417 for $C_{39}H_{54}O$; M−120 (M−C_8H_8O), measured *m/e* 462.385, calc. 462.386 for $C_{33}H_{50}O$; M−172 (M−$C_{12}H_{12}O$), measured 410.353, calc. 410.355 for $C_{29}H_{46}O$.

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