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Bacterial Carotenoids XXIV*

The Carotenoids of Thiorhodaceae 7.* Cross-conjugated Carotenals

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The structures of the carotenoids warmingol, anhydro-warmingone, warmingone, and P500 isolated from Thiorhodaceae spp. have been investigated by spectroscopic (electronic-, infrared, mass, and proton magnetic resonance spectra) and chemical methods.

The last three carotenoids represent a class of cross-conjugated carotenals in which one of the lateral methyl groups of the polyene chain is formally oxidized to an aldehyde group.

Anhydro-warmingone is considered to be a lycopenal, 13-*cis*-lycopen-20-al (*2a*) or 9-*cis*-lycopen-19-al (*2b*); warmingone a rhodopinal, 13- or 13′-*cis*-rhodopin-20- or 20′-al (*4a*) or 9- or 9′-*cis*-rhodopin-19- or 19′-al (*4b*); and P500 a tetrahydro-spirilloxanthinal, 13-*cis*-3,4,3′,4′-tetrahydro-spirilloxanthin-20-al (*17a*) or 9-*cis*-3,4,3′,4′-tetrahydro-spirilloxanthin-19-al (*17b*). Warmingol is the rhodopinol (*5a* or *b*) corresponding to rhodopinal (*4a* or *b*). Their previous trivial names should now be abandoned.

Twenty-one derivatives have been prepared from the new carotenals. They appear to have stable *cis*-configuration and exhibit peculiar electronic spectra.

The carotenoids produced in the ultimate biosynthetic steps by photosynthetic purple sulphur bacteria may be divided into three groups (i) the normal spirilloxanthin series, (ii) okenone, and (iii) the warmingone series.[1] The structures of the carotenoids belonging to the normal spirilloxanthin series are well established.[1,2] Also the structure of okenone has recently been elucidated.[3] However, the structures of warmingone and related compounds have remained undetermined.

In addition to lycopene (*1*) and rhodopin (*3*), the warmingone series comprises three carotenoids, Pigments 1, 2, and 3, first isolated from *Chromatium warmingii* Migula.[4] The main carotenoid, Pigment 2, was considered to be a conjugated ketone and designated warmingone. Hydride reduction of warmingone resulted in Pigment 3,[4] later referred to as warmingol.[2,1] In subsequent

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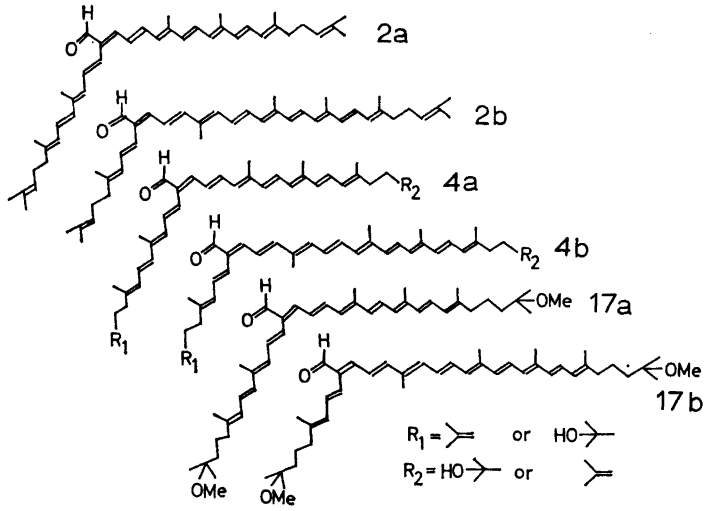
dehydration studies warmingone was converted to Pigment 1, accordingly referred to as anhydro-warmingone.[2,1]

In the present work warmingone and anhydro-warmingone are shown to represent a class of cross-conjugated carotenals with C_{40} skeletons, in which a lateral methyl group has been formally oxidized to an aldehyde group. The structures (*2a* or *b* and *4a* or *b*) proposed for the new carotenals can be more precisely derived from rhodopin (*3*) and lycopene (*1*), and it is now suggested that their previous trivial names should be abandoned.

The minor carotenoid, P500, from a *Thiococcus* sp. strain RG3 [5–7] belongs to this same class of carotenals and is assigned the methoxylated structure *17a* or *b*. The numbering used for the methyl groups is in accordance with the IUPAC recommendations.[8]



As a source of the carotenoids of the "warmingone" series cells of *Chromatium warmingii* strains Ostrau and Melbourne as well as a *Thiocystis* sp. isolated by Eimhjellen [9] from the marine sponge *Halichondria panicea* were used. The carotenoid compositions of these *Thiorhodaceae* spp. are given in Table 1. The *Thiocystis* sp. was more easily cultivated and provided the major part of the pigments studied.

Lycopene (*1*) and rhodopin (*3*) had previously been identified.[4] The identification of rhodopin (*3*) was confirmed by a mixed melting point determination with authentic rhodopin and comparison of infrared and NMR-data with those of authentic *3*. Pure crystalline lycopene (*1*) could not be isolated because of the presence of colourless contaminants.

Until their structures are unequivocally established the main carotenoid ("warmingone") will be referred to as rhodopinal (*4a* or *b*) and the naturally occurring corresponding allylic alcohol ("warmingol") as rhodopinol (*5a* or *b*).

Table 1. Carotenoid composition of the *Thiocystis* and *Chromatium* strains studied.

Carotenoid	% of total carotenoid			
	Chromatium warmingii			
	Strain Lot 1	Melbourne Lot 2	Strain Ostrau	*Thiocystis* sp.
Lycopene (*1*)	13	4	4	4
Lycopenal (*2*)	1	1	1	3
Rhodopin (*3*)	17	11	12	31
Rhodopinal (*4*)	48	60	65	57
Rhodopinol (*5*)	21	24	18	5
Total carotenoid in % of dry weight	0.41	0.28	0.36	0.22
Cells (dry weight) in g investigated	0.3	30	18	204

Crystalline lycopenal ("anhydro-warmingone", *2*), rhodopinal (*4*), and rhodopinol (*5*) could not be obtained. Judging from NMR-spectra, chromatographic fractions containing apparently pure rhodopinal (*4*) were isolated. The resistance towards crystallization therefore seems to be a characteristic feature of these compounds, rather than a phenomenon caused by accompanying impurities.

The aldehydic character of rhodopinal (*4*) was indicated by the ease with which it underwent the Wittig reaction (see below),[5,10] and by formation of the corresponding dimethyl acetal (*6*), 2,4-dinitrophenylhydrazone (*7*), and oxime (*8*), as well as IR-absorption (see Fig. 1) in chloroform at 2760 and 1685 cm^{-1} (conjugated aldehyde), and was confirmed by the NMR-spectrum (aldehydic proton at 0.5 τ). Rhodopinal (*4*) gave no acetate under standard acetylation conditions, and its hydride reduction product, rhodopinol (*5*), gave a monoacetate (*9*) only. No intermediates were detected during formation of the aldehyde derivatives *6*, *7*, and *8*. Hence, only a single carbonyl function

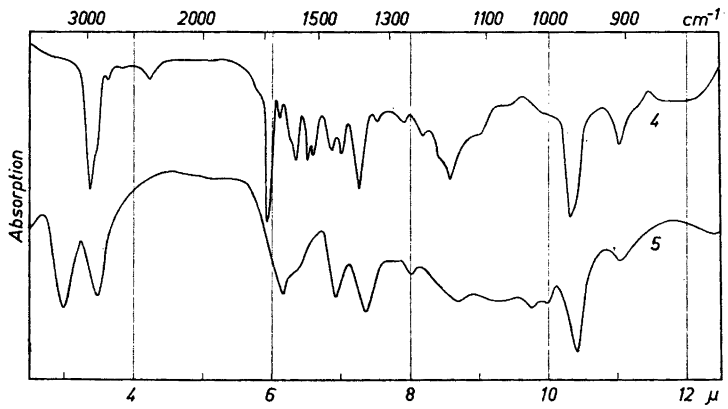


Fig. 1. Infrared spectra of rhodopinal (*4*) in chloroform and rhodopinol (*5*) in KBr pellet.

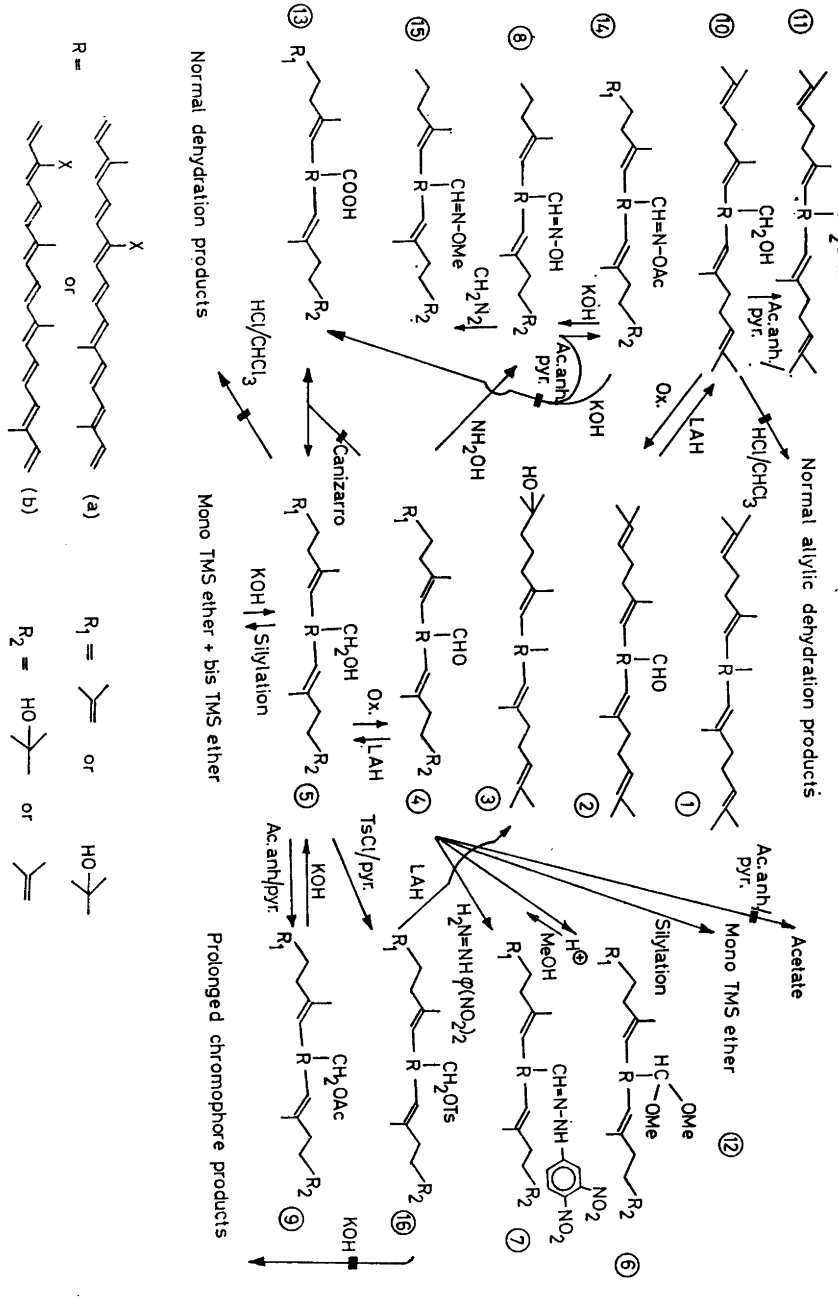
is present in rhodopinal (*4*). The same inference can be drawn for the lycopenal (*2*) which on hydride reduction gave a mono-ol (*10*) which in turn was converted to a monoacetate (*11*).

The presence of a tertiary hydroxyl group in rhodopinal (*4*) and rhodopinol (*5*) was indicated by IR-absorption at 1140 and 905 cm^{-1} (see Fig. 1), and by the partition behaviour of rhodopinol (*5*) and rhodopinyl acetate (*9*), and was verified by preparation of its trimethylsilyl ether (*12*) [11] as well as dehydration with phosphorus oxychloride in pyridine [12] to a product identical with the natural lycopenal (*2*). The latter reaction suggested the presence of a hydroxylated end-group as in rhodopin (*1*). This was supported by a sharp NMR-signal at 8.76 τ.[13]

Evidence of other functional groups could not be obtained. Methoxyl substituents and aryl end-groups were ruled out from IR- and NMR-spectra (Figs. 1 and 2). The second end-group was considered to be isopropylidene, judging from the presence of signals at 8.32 and 8.39 τ.[13]

Further experiments were carried out in order to gain information about the location of the aldehyde group. Since the aldehydic proton caused a singlet in the NMR-spectrum the aldehyde group was attached to a tertiary carbon atom. Attempts were therefore made to obtain from rhodopinal (*4*) the corresponding carboxylic acid (*13*) by a Cannizzaro reaction. The acid (*13*) could not be obtained either in this way or by a different approach according to the method employed by Kuhn and Grundmann [14] *via* the oxime (*8*) and the corresponding nitrile. Treatment of rhodopinaldoxime (*8*) with acetic anhydride, under conditions where the carotenoid survived, furnished no nitrile and gave exclusively the acetate (*14*), which on alkali treatment reverted to the oxime (*8*). Re-acetylation of the hydrolysis product again gave the oxime acetate (*14*). It should be mentioned that a consideration of R_F-values and partition ratios only in this case is misleading, since the product obtained after alkali treatment of the presumed nitrile exhibited weak acidic properties and could even be methylated with diazomethane. The product, considered to be *15*, could be mistaken for the methyl ester of *13*. It is noteworthy that β-apo-2'-carotenyl (C_{37}) oxime studied as a model compound behaved similarly. It is recognized that only *anti* oximes (or their acetates) undergo elimination to nitriles.[15] It may therefore be inferred that *syn* oximes exclusively were formed on oximation of rhodopinal (*4*) and β-apo-2'-carotenal (C_{37}), and that conditions permitting isomerization to the *anti* isomer and subsequent dehydration were not achieved. The electronic spectra of the derivatives described above will be discussed later.

Although the oxidation attempts failed, reduction of rhodopinal (*4*) to rhodopinol (*5*) was easily accomplished with lithium aluminium hydride.[4] The reduction caused a large hypsochromic shift, confirming conjugation of the aldehyde group with the polyene chain. As already mentioned the lycopenal (*2*) could be reduced in the same manner to the corresponding lycopenol (*10*). In spite of the allylic character of the primary hydroxyl groups of the two reduction products (*5* and *10*), both alcohols failed to give normal allylic dehydration products on treatment with acidified chloroform.[16] Despite the readiness with which this reaction yields anhydro-vitamin A from vitamin A,[17] the synthesis of vinylidene compounds does not seem to be favoured for


Normal allylic dehydration products
Normal dehydration products
Mono TMS ether + bis TMS ether
Prolonged chromophore products
Mono TMS ether
Acetate
Silylation
Ac.anh/pyr.
HCl/CHCl3
LAH
Ox.
KOH
NH2OH
Canizarro
TsCl/pyr.
MeOH
H2N=NHφ(NO2)2
R = or
R1 = or
R2 = HO or

this class of carotenols, as was the case with β-apo-2′-carotenol (C_{37}).[18] Alkali treatment of rhodopinyl tosylate (*16*) did not result in any elimination.

Rhodopinol (*5*) could be reversibly oxidized to rhodopinal (*4*), thus proving that no rearrangement had occurred during reduction of *4* to *5*. However, the yields of the allylic oxidation reaction were unexpectedly low. Rhodopinol (*5*) was readily destroyed by *p*-chloranil [19] or 2,3-dichloro-5,6-dicyanobenzoquinone,[20] whereas treatment with silver oxide [21] or air in the presence of iodine and light,[19] promoted transformation to rhodopinal (*4*). Under the last conditions oxidation of the lycopenol (*10*) to the lycopenal (*2*) was also observed.

The close chemical relationship between rhodopinal (*4*) and rhodopin (*3*) was established by reduction of rhodopinyl tosylate (*16*) with lithium aluminium hydride which gave rhodopin (*3*) in low yield.[22] That rhodopinal (*4*) represents a derivative of rhodopin (*3*) in which a lateral methyl group is formally oxidized to an aldehyde group was also confirmed by mass spectrometric determination of the molecular weight of its 2,4-dinitrophenylhydrazone (*7*), the only crystalline derivative of rhodopinal (*4*). Moreover, the electronic spectrum of rhodopinol (*5*, Fig. 6) was indicative of a chromophore of 11 spectroscopically efficient carbon-carbon double bonds.[2] Concerning the location of the presumed undecaenal chromophoric system, the NMR-spectra of rhodopinal (*4*, Fig. 2) and rhodopinal 2,4-dinitrophenylhydrazone (*7*, Fig. 3) gave useful information. Whereas the spectrum of *4* was not suitable for measurement of intensity ratios of the methyl signals the spectrum of *7* was consistent with the presence of two end-of-chain- and only three in-chain methyls. This evidence suggested that one of the four central methyl groups in rhodopin (*3*) was replaced by an aldehyde group in rhodopinal (*4*).

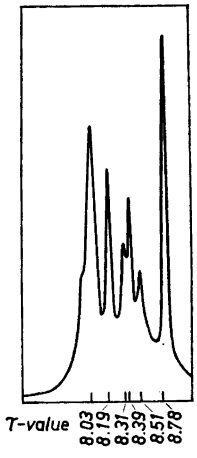


Fig. 2. Methyl region of proton magnetic resonance spectrum at 60 Mc/sec of rhodopinal (*4*) in deuterochloroform.

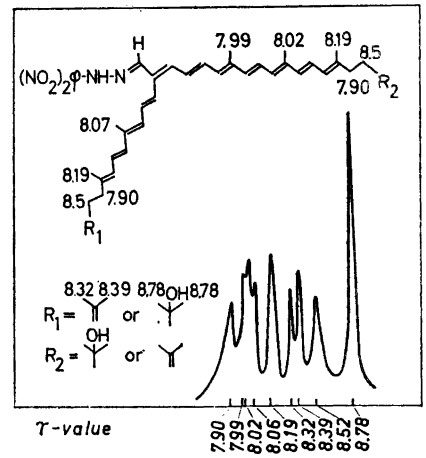
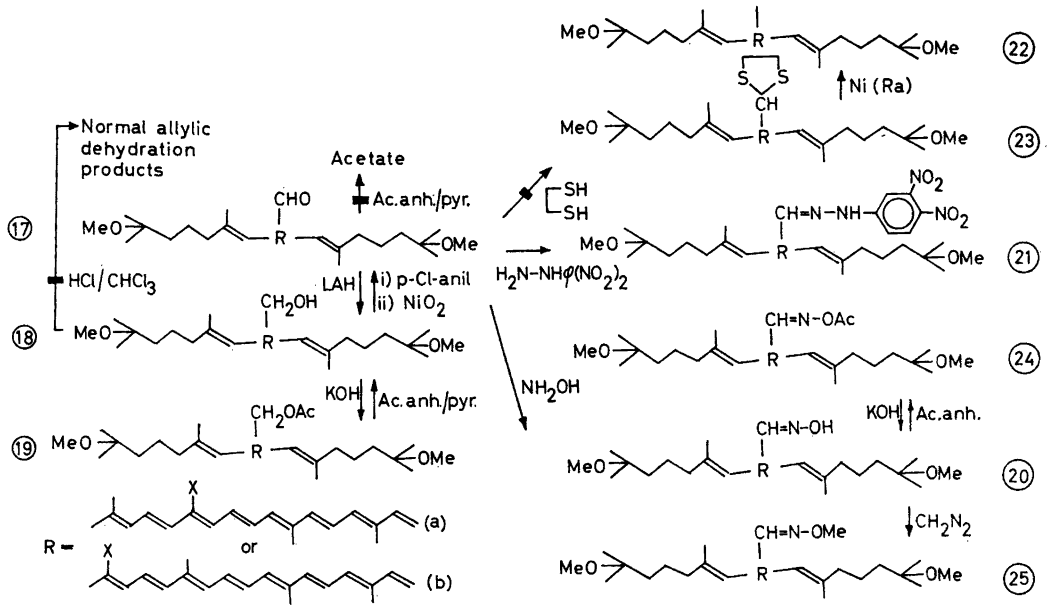


Fig. 3. Methyl region of proton magnetic resonance spectrum at 100 Mc/sec with tentative signal assignments of rhodopinal 2,4-dinitrophenylhydrazone (*7*) in deuterochloroform (5 repeated integrated scans).



In addition to the two new carotenals (*2* and *4*) discussed above a third representative provisionally called P500 of this class was recently isolated from a *Thiorhodaceae* sp. [5,6] now considered to be a *Thiococcus* sp.[7] The structure 3,4,3′,4′-tetrahydro-spirilloxanthin-20- or 19-al (*17a* or *b*) may now be ascribed to P500. Also this carotenal was non-crystalline. The electronic spectra of the natural pigment (*17*) and of the derivatives described below (*18*—*21* and *24*, *25*) were identical with those of the corresponding derivatives of the lycopenal (*2*) and rhodopinal (*4*). However, the adsorptive properties of natural *17* were intermediate between those of *2* and *4*. Natural *17* was resistant towards acetylation. Hydride reduction provided a product (*18*) with a partition ratio and adsorptive properties characteristic of a mono-ol (*18*). On acetylation this mono-ol (*18*) gave a monoacetate (*19*) with low polarity indicating the absence of further free hydroxyl groups. By inference it was concluded that hydroxyl groups were absent and a single carbonyl group was present in *17*. Natural *17* readily condensed with $R—NH_2$ derivatives giving the corresponding mono-oxime (*20*) as well as a crystalline 2,4-dinitrophenylhydrazone (*21*). The IR-spectrum of *21* exhibited a strong intensity absorption band at 1080 cm^{-1}, also present in natural *17* (see Fig. 4). The intensity ratios of the 1080 cm^{-1} band relative to the *trans* disubstituted double bond absorption at 955 cm^{-1}, compared with that for 3,4,3′,4′-tetrahydro-spirilloxanthin (*22*) [5] suggested the presence of more that one methoxy group in the natural pigment, thus indicating structure *17* for P500. Mass spectrometric determination of the molecular weight of the 2,4-dinitrophenylhydrazone (*21*) indeed confirmed this supposition. Further support was sought by attempting trans-

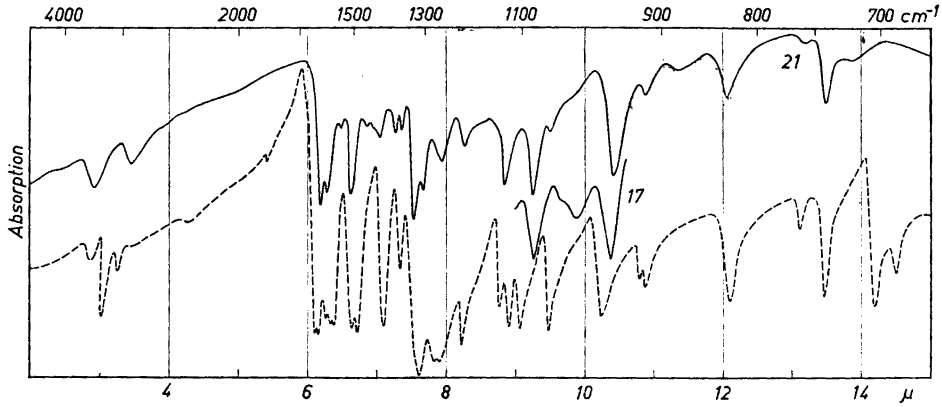


Fig. 4. Infrared spectra, recorded in KBr pellet of 3,4,3′,4′-tetrahydro-spirilloxanthinal 2,4-dinitrophenylhydrazone (*21*), 3,4,3′,4′-tetrahydro-spirilloxanthinal (*17*) and - - - - 2,4-dinitrophenylhydrazine.

formation of natural *17* to *22 via* its thioketal (*23*) followed by reduction.[23] However, under the conditions employed, thioketal formation did not occur.

As found with *2* and *4*, the corresponding acid could not be obtained from *17*. Treatment of the oxime (*20*) with acetic anhydride resulted in the oxime acetate (*24*) which on alkali treatment reverted to *20*, which was reacted with diazomethane to a product considered to be *25*.

The general reaction behaviour described above and the appearance of the electronic spectra of the corresponding derivatives were thus taken as demonstrating identical chromophoric systems in *2*, *4*, and *17*. Together with the mass spectrometric and IR-information, supporting the presence of two methoxyl groups in P500, the structure 3,4,3′,4′-tetrahydro-spirilloxanthin-20- or 19-al (*17 a* or *b*) was deduced for this minor pigment from *Thiococcus*.

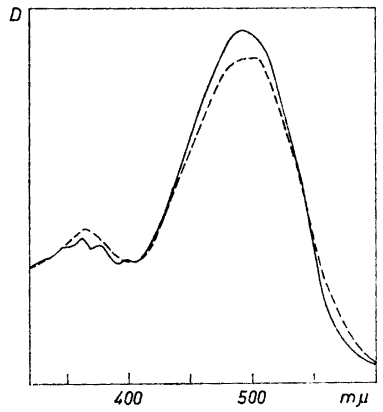


Fig. 5. Absorption spectrum in visible light of natural rhodopinal (*4*) in ——— petroleum ether and - - - - acetone.

The fact that *17* occurs in this bacterium together with 3,4,3′,4′-tetrahydrospirilloxanthin (*22*) [5,6] lends some further support to this conclusion.

The exceptional appearance of the visible light absorption spectrum of rhodopinal (*4*, Fig. 5) has already been pointed out.[4,24] The electronic spectra of the carotenals *2*, *4*, and *17* differ remarkably from those of the apo-carotenals synthesized by Isler and co-workers.[25,26] As seen from Fig. 5 the main, naturally occurring stereoisomer of rhodopinal (*4*) exhibits strong absorption in the 360 mμ region, suggesting the presence of sterically nonhindered *cis* double bond(*s*).[27] However, iodine catalyzed isomerization in light did not result in the spectral changes typical of *cis-trans* isomerization, and no isomer with all-*trans* characteristics (lack of *cis*-peak, increase in fine-structure and wavelength position of the fundamental bands in the electronic spectrum [27]) was formed.[4] The observed behaviour confirmed the absence of sterically hindered *cis* double bonds, since such hindered *cis*-isomers are known to isomerize irreversibly to all-*trans* (and unhindered *cis*-isomers) in the presence of iodine.[27] However, the result does not exclude the case hitherto unreported in the carotenoid series that a particular *cis* configuration is thermodynamically more stable than the all-*trans* form. Indeed the resistance of these carotenals towards crystallization is also suggestive of a *cis* configuration.

Hydride reduction of the carotenals, *e.g. 17*, which in our experience generally does not promote extensive *cis*-isomerization of carotenoids, resulted in conversion to the alcohol *18*, consisting of more than 90 % of a stereoisomer with an intense double *cis*-peak (see Fig. 6). Iodine catalyzed isomerization of this stereoisomer gave the presumed *trans* isomer (see Fig. 6), although this was present only to an extent of *ca.* 20 % of the stereomutation mixture. Similar observations were made on reduction of rhodopinal (*4*) and the lycopenal (*2*), indicating that a *cis* double bond was also originally present in the carotenals *2*, *4*, and *17*. Since stable *cis*-carotenoids of the type described above have previously not been encountered, it appears that their stability is governed by the hetero (cross-conjugated) substituent on the polyene chain. Hence it

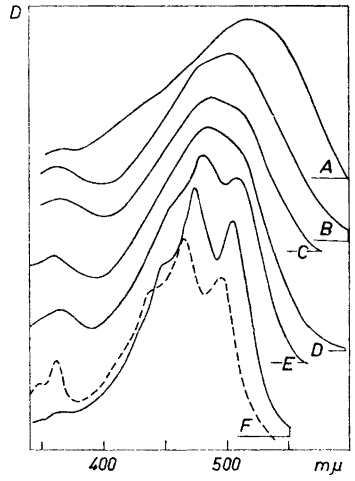


Fig. 6. Absorption spectra in visible light of various derivatives of 3,4,3′,4′-tetrahydro-spirilloxanthinal (*17*) in acetone, ——— stereoisomer absorbing at longest wavelength, - - - - neo A.

A 2,4-dinitrophenylhydrazone (*21*),
B aldehyde (*17*),
C oxime acetate (*24*),
D oxime ether (*25*)
E oxime (*20*),
F alcohol (*18*).

may be deduced that the *cis* double bond is directly connected to this substituent. The *cis-trans* equilibria of rhodopinal (*5*) and rhodopinylidenacetaldehyde (*29*) described below are taken to support this conclusion.

According to Zechmeister [27] intense *cis*-peaks of the type observed are exhibited by strongly bent carotenoids possessing near-to-central *cis* double bond. On this basis our results can be best accomodated with the *cis* bond in the 13-position (or 13′ in the case of *4* and *5*) and the aldehyde group in 20 (or 20′ for *4*), *i.e.* with the structures *2a*, *4a*, and *17a*. Some support for the 20- (or 20′)-al formulation is derived from the NMR-spectrum of rhodopinal 2,4-dinitrophenylhydrazone (*7*). The chemical shifts of two of the in-chain methyls differ relative to those of rhodopinal (*4*). This effect is presumably due to the influence of the phenyl substituent;[28] for tentative signal assignments see Fig. 3.

However, the *2b*, *4b*, and *17b* structures cannot definitely be ruled out, and the final establishment of the structures of the new carotenals should be sought by total synthesis. The position of the aldehyde group in *4* and the primary hydroxyl group in *5* relative to the hydroxylated end-group must also be determined by synthesis.

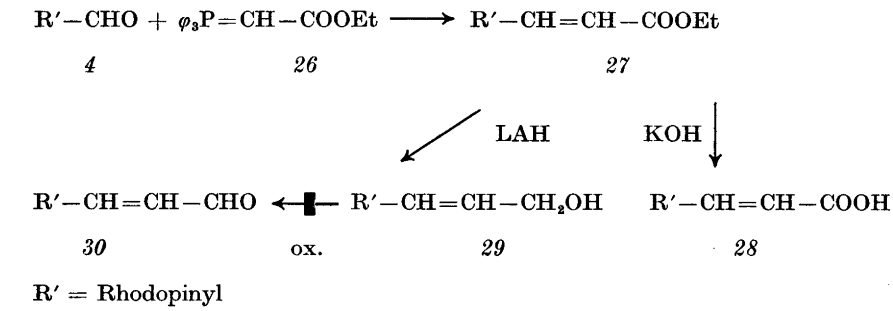
The exceptional shape of the electronic spectra of the new carotenals may be explained from these cross-conjugated structures. In petroleum ether solution (Fig. 5) the double *cis*-peak of the *cis*-undecaene system is recognizable at 345 and 362 mμ. In addition a second double *cis*-peak at 378, 398 mμ may be attributed to the cross-conjugated *cis*-undecaenal chromophore. The broadness of the fundamental band is considered to be the effect of cross-conjugation, comprising at least two effective chromophores. The remarkable bathochromic effect obtained by this type of cross-conjugation may also be seen from the data recently published by Haeck and Kralt [29] for some carotenoid-like compounds.

By an empirical rule [27] *cis*-peaks in carotenoids are expected to occur in petroleum ether solution at 142 ± 2 mμ lower wavelengths than the maximum at the longest wavelengths in the *trans* isomer. The aldehyde derivatives described here, the 2,4-dinitrophenylhydrazones (*7* and *21*), the oximes (*8* and *20*), the oxime acetates (*14* and *24*), and oxime ethers (*15* and *25*) do not appear to obey this rule (see Fig. 6). In spite of a marked change in wavelength position of the fundamental band, the apparent *cis*-peak is located at a constant position identical with that of the corresponding allylic alcohols (*5*, *10*, and *18*) or acetal (*6*). The *cis*-polyene chromophore does not seem to be effected by substitution of the crossconjugated aldehyde group. In the case of the 2,4-dinitrophenylhydrazone (see *21*, Fig. 6) an inflexion is seen in addition at the expected *cis*-peak position for the cross-conjugated chromophore.

All the above derivatives exhibit strong absorption in the *cis*-peak region, taken to manifest the higher stability of the *cis*-configuration in these cases. In fact, only in the case of the allylic alcohols (*5*, *10*, and *18*) could the *trans* isomers be isolated, although even in this case the *cis* isomer was the more stable.

In order to obtain some more information about the structural features governing the stereochemistry and electronic spectra of such compounds,

rhodopinal (*4*) was condensed with carbethoxymethylenetriphenyl phosphorane (*26*) in a Wittig reaction. The resulting ester (*27*) was converted to the free acid (*28*) by saponification. The corresponding primary alcohol (*29*) was obtained on hydride reduction of the ester (*27*), but attempts to oxidize *29* to the corresponding aldehyde (*30*) failed; *cf.* resistance of rhodopinal (*4*) towards oxidation.



R′ = Rhodopinyl

As seen from Fig. 7, the alcohol (*29*) absorbs light at longer wavelengths than does rhodopinol (*5*). The fine-structure in the spectrum is less pronounced than expected for an aliphatic dodecaene,[2] and in this case no *trans* isomer could be isolated, the isomerization of the *cis* double bond presumably being prohibited by the larger lateral substituent. The ester (*27*) and free acid (*28*) exhibit similar types of spectra (see Fig. 7) to the natural carotenals. Their spectra are shifted to somewhat shorter wavelengths relative to rhodopinal (*4*), and it is interesting to note the decrease in efficiency of cross-conjugation of various substituents in the system under discussion (see Table 2).

In summary, the evidence presented is taken to support the cross-conjugated formulations *2a* or *b*, *4a* or *b*, and *17a* or *b* for the new carotenals.

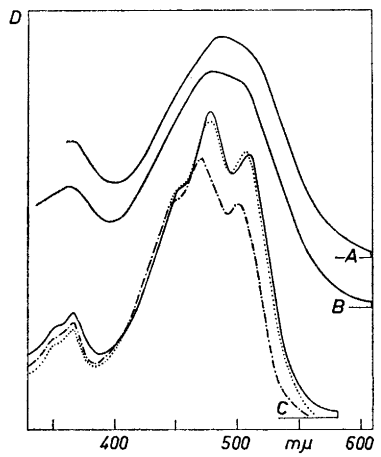


Fig. 7. Absorption spectra in visible light in acetone of ——— stereoisomer absorbing at longest wavelength, ····· neo A and ——·—— neo B.

A Ethyl rhodopinylidenacetate (*27*),
B Rhodopinylidenacetic acid (*28*),
C 2-Rhodopinylidene ethanol (*30*).

Table 2. Bathochromic effect (Δ) of various cross-conjugated groups in the aliphatic 13-*cis*-20- or 9-*cis*-19-x-undecaene carotenoid chromophore.

Cross-conjugated group (x)	In acetone (mμ) λ_{max}	Δ
$-CH=N-NH\text{-}2,4\text{-}(NO_2)_2\varphi$	516	50
$-CH=O$	503	37
$-CH=CH-COOCH_3$	489	23
$-CH=N-OCH_3$	486	20
$-CH=N-O-COCH_3$	485	19
$-CH=CH-COOH$	483	17
$-CH=N-OH$	481	15
$-CH=CH-CH_2OH$	478	12
$-CH_2OH$	466	—

Rhodopinol (*5*) is the first known naturally occurring carotenoid where a primary hydroxyl group has replaced one of the lateral methyl groups on the polyene chain. The preference for *cis*-configuration in such carotenols may facilitate the identification of this structural feature in the future.

The biochemical implications of the structural determinations of the hitherto unknown members of the rhodopinal ("warmingone") series, *2*, *4*, and *5*, as well as of tetrahydrospirilloxanthinal ("P500"; *17*), will be discussed elsewhere.

EXPERIMENTAL

Materials and general methods. When not stated to the contrary these were as summarized in an earlier paper of this series.[30] Column chromatography was generally performed on neutral alumina, activity grade 2. Adsorptive properties including R_F-values on kieselguhr paper are compiled in Table 3. Chemical reactions were followed by periodical paper-chromatographic examinations. The terms in which spectral characteristics are described have been defined elsewhere.[31] If not specified, chemical reactions were carried out at room temperature. Silylation was effected according to the procedure described elsewhere.[11]

Biological material. Chromatium warmingii Migula strains Melbourne and Ostrau, a *Thiocystis* sp. isolated from a marine sponge *Halichondria panicea* by Eimhjellen,[9] and a *Thiococcus* sp., strain RG3, also isolated by Eimhjellen,[7] were used. The two former strains were cultivated in Institut für Mikrobiologie der Universität, Göttingen, by the courtesy of Professor N. Pfennig, and the two latter organisms were cultivated by Docent K. E. Eimhjellen in the Department of Biochemistry, this University, according to the methods described elsewhere.[1,4,7,9]

Pigment extraction, saponification and chromatographic separation. The *Chromatium* and *Thiocystis* spp. were extracted at room temperature with successive portions of acetone-methanol (3:1) until a brownish cell residue, from which no further pigment was released with chloroform or carbon disulphide, was obtained. Between each extraction centrifuging at 6000 *g* was carried out. The acetone-methanol extract was concentrated to about 500 ml and the pigments transferred to benzene (or ether) in the usual manner.

Saponification of aliquots of the pigment mixtures, followed by quantitative paper-chromatographic and spectrophotometric examination of the carotenoid components, revealed the absence of alkali-labile carotenoids. Saponification under standard conditions (5 % KOH-methanol, 1 h, room temperature) was consequently included in the purification procedure. The carotenoids were transferred to benzene or ether in the usual manner. In the former case dehydration of the extracts with anhydrous sodium sulphate could

be omitted. The carotenoids were chromatographed on neutral alumina, activity grade 2, *cf.* Ref. 4. The pigment yields and composition of the carotenoid mixtures are given in Table 1. For *1* and *3* values of $E^{1\%}_{1\,cm} = 3000$, and for *2*, *4*, and *17* $E^{1\%}_{1\,cm} = 2300$ at λ_{max} in petroleum ether, were used.

Isolation of P500 from the *Thiococcus* sp. has been described elsewhere.[5,6]

The identity of samples of lycopene (*1*), lycopenal (*2*), rhodopin (*3*), rhodopinal (*4*) and rhodopinol (*5*) isolated from the various biological sources was secured by co-chromatography tests. Some of the other data reported below were also obtained for a particular compound orginating from different sources.

Lycopenal (*2*)

Crystallization failed.

Absorption spectrum in visible light. This was superimposable on that of *4*; see Table 2 and Fig. 5.

Stereoisomerization. On iodine catalysis in light the natural stereoisomer was partly converted to a neo A (40 %; 363 and 499 mμ) and a neo U (5 %; 363 and 493 mμ) isomer. Relative abundance in the equilibrium mixture and absorption maxima in acetone are given in parenthesis.

Lycopenol (10). *2* in dry ether was reduced with lithium aluminium hydride in the usual manner; pigment recovery was 85–95 %. *10* was purified by column chromatography. The iodine catalysed eguilibrium mixture comprised three stereoisomers, neo B (362, 450, 470, and 501 mμ), neo A (362, 441, 465, and 494 mμ) and *trans* (362, 450, 474, and 503 mμ). Absorption maxima in acetone are given in parenthesis.

In one iodine catalysis experiment the formation of *2* was observed.

Treatment of lycopenol (10) with acid chloroform. 10 (0.05 mg) was treated with 0.025 N HCl–$CHCl_3$ reagent for 5 min; pigment recovery was 95 %. More yellow products only were formed. Longer reaction periods resulted in considerable pigment loss.

Lycopenol acetate (11). Standard acetylation of *2* (0.1 mg) in dry pyridine (0.5 ml) with acetic anhydride (0.1 ml) gave *11*, which on alkali treatment (5 % KOH in methanol) reverted to *10*.

Rhodopin (*3*)

3 crystallized from acetone-petroleum ether in red needles of m.p. 170°C, which was undepressed (m.p. 171–172°C) on admixture with an authentic [13] sample (m.p. 172.5°C). The present pigment had abs. max. at 362, (422), 448, 474 ($E^{1\%}_{1\,cm}$ = *ca.* 3000), and 506 mμ, % III/II = 76 in acetone. The IR-spectrum (KBr pellet) had abs. max. at 3450, 2940, 1638, 1552, 1445, 1380, 1365, 1185, 1145, 952, 905, and 821 cm^{-1}, and agreed completely with that of authentic *3*. The NMR-spectrum recorded in $CDCl_3$ at 60 Mc/sec had signals at 7.85 (6), 8.03 (12), 8.19 (6), 8.32 (3), 8.39 (3), 8.51 (4), and 8.76 (6) τ. Relative integrals are given in parenthesis. The spectrum agreed well with that previously reported for *3*.[13]

Rhodopinal (*4*)

Crystallization attempts. Crystalline *4* could not be obtained from a number of solvent pairs. Re-chromatographed and twice precipitated (acetone-petroleum ether) *4* afforded a black substance (m.p. unsharp around 195°C) on the glass wall in low yield. Non-crystalline *4*, twice chromatographed on alumina and paper-chromatographically homogeneous was used in subsequent experiments.

General stability. Amorphous *4* appeared especially unstable. *4* was best stored in fairly dilute solutions under nitrogen at a low temperature. In concentrated solutions particularly yellow, strongly adsorbed decomposition products were formed. Paper chromatograms faded unusually rapidly when stored in air. Addition of KOH (0.2 %) or glacial acetic acid (0.2 %) had no effect on the absorption spectrum in acetone.

Absorption spectrum in visible light. Natural, paper-chromatographically homogeneous *4* exhibited abs. max. in acetone at 365 and 505 mμ and in petroleum ether at (358), 363, 378, 398, and 495 mμ (see Fig. 5).

IR-spectrum. Amorphous samples (KBr pellet) had abs. max. at 3410, 2980, 1670, 1600, 1450, 1380—1368, 1300, 1260, 1175 (broad), 970, 905, and 803 cm^{-1}. However, the recovery of *4* from the KBr pellet was very low. In chloroform solution (10 % sample) abs. max. were located at 2960 (CH); (2900) and 2760 (aldehydic CH); 1685 (conj. aldehyde); 1640, 1585, 1538, 1515 (conj. double bonds); 1448 (CH_2); 1428; 1380 (methyl); 1325; 1165 (*tert.* OH?); 970 (*trans* disubst. double bonds) and 905 (C—C(—C)—O—) cm^{-1}, see Fig. 1.

NMR-spectrum. The spectrum, recorded in $CDCl_3$ at 60 Mc/sec had signals at τ-values 0.5 (aldehyde); 3.3—4.4 (olefinic H); 8.0—8.03 (in chain methyls); 8.19 (end-of-chain methyls); 8.32, 8.39 (isopropylidene); 8.50 (CH_2) and 8.78 (*gem* methyl at tert. oxygen substituent); see Fig. 2.

Mass spectrum. The determination failed.

Partition tests. In petroleum ether/95 % methanol *4* had a partition ratio of 48:52, unchanged upon addition of 0.2 % KOH.

Stereoisomerization. The reversible formation of a neo U isomer, absorbing light at shorter wavelengths, on iodine catalysis in light, has already been reported.[4] Other stereoisomers could not be isolated.

Attempted ω,ω'-hydrogenation.[32] The procedure used for 2,2'-diketospirilloxanthin [33] was followed. To *4* (0.55 mg) in dry pyridine (3 ml) glacial acetic acid (0.5 ml) and a spatula tip of zinc powder were added. After 8 min no colour change had occurred. The reaction mixture contained *4* only; pigment recovery was 85 %.

Attempts at a Cannizzaro reaction. Treatment of *4* with 10 % KOH in methanol at room temperature or 45°C for 2—24 h gave 20—7 % pigment recovery, but no formation of *5* and *13*.

Rhodopinal dimethyl acetal (6). To *4* (2 mg) in methanol (1 ml) conc. aqueous hydrogen chloride in methanol (1 %, 0.1 ml) was added. The reaction mixture immediately turned orange, and after 1 min the pigments were transferred to ether in the usual manner; pigment recovery was 80 %. Paper chromatography revealed the presence of *4* (20 %) and *6* (80 %). The product (*6*) had abs. max. at 345, 362, 440, 466, and 492 mμ in acetone; % $D_B/D_{II} = 48$.

On treatment of the acetal (*6*) with hydrogen chloride in methanol as above, the reversible formation of *4* was observed after 1 min.

Rhodopinal 2,4-dinitrophenylhydrazone (7). To *4* (15 mg) in glacial acetic acid (5 ml) 2,4-dinitrophenylhydrazine (25 mg) was added. A quantitative conversion to *7* was observed after 20 min. The pigment was transferred to petroleum ether, and this extract was washed with aqueous sodium bicarbonate; pigment recovery was 93 %. After column chromatography *7* was crystallized from acetone-petroleum ether and the melting point recorded as 175—180°C. Absorption data in visible light are given in Table 2, $E^{1\,\%}_{1\,cm} = 1245$ at λ_{max} in acetone. The spectral curve corresponded to that of *21* (Fig. 6). The IR-spectrum (KBr) had abs. max. at 3420, 2930, 1620, 1595, 1515, 1430, 1325, 1298, 1265, 1215, 1133, 1080, 1052, 962, 918, 880, 840, and 740 cm^{-1}. In Fig. 3 is given the NMR-spectrum with assignments, recorded in $CDCl_3$ at 100 Mc/sec. The mass spectrum showed the molecular peak at $m/e = 748$ and had a continuous series of peaks at lower m/e presumably caused by thermal decomposition.

Rhodopinaldoxime (8). To *4* (19.7 mg) in dry benzene (20 ml) and dry pyridine (5 ml) hydroxylamine hydrochloride (100 mg) was added. The mixture was kept at 40°C for 30 min, and then an additional amount of hydroxylamine hydrochloride (100 mg) was added. After 4 h the reaction mixture comprised *4* (25 %) and *8* (25 % + 50 % in two zones). The pigments were transferred to benzene in the usual manner and submitted to column chromatography. *4* (16 %) and *8* (70 %; requiring an eluent of 10 % methanol in ether) were recovered. The aldoxime (*8*) could not be crystallized, and provided a sticky precipitate from acetone-petroleum ether or ether-petroleum ether only. Absorption data in visible light are given in Table 2. The spectrum corresponded to that of *20* in Fig. 6. The IR-spectrum (KBr pellet) had abs. max. at 3450, 2940, 1620, 1560, 1450, 1380, 1140, 1045, and 960 cm^{-1}.

In a second similar experiment *4* (0.5 mg) was quantitatively converted to *8* in 1 h.

Rhodopinaldoxime acetate (*14*). To *8* (0.4 mg) in dry ether (1 ml) acetic anhydride (1 ml) was added. After 16 min at 50°C a complete conversion to *14* was observed. The pigment was transferred to benzene in the usual manner; pigment recovery was 81 %. Absorption maxima in visible light are given in Table 2. The spectrum corresponded to that of *24* in Fig. 6.

When *8* in glacial acetic acid was boiled under reflux according to the method of Kuhn and Grundmann [14] the carotenoids were destroyed after 35 min. No acid (*13*) was detected by paper chromatography at any stage.

Treatment of *14* (0.3 mg) with 8 % KOH in methanol for 2 min resulted in quantitative formation of the aldoxime (*8*) judged by absorption spectra in visible light and co-chromatography tests. Re-treatment of the saponification product (*8*) with acetic anhydride again gave *14*.

Acetylation. Standard acetylation of *4* (0.5 mg) in dry pyridine (2 ml) and acetic anhydride (0.2 ml) for 23 h resulted in 72 % pigment recovery. The reaction mixture contained exclusively unreacted *4*.

Rhodopinal trimethylsilyl ether (*12*). Treatment of *4* (0.34 mg) in dry pyridine (0.5 ml) with hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) for 1 h resulted in quantitative formation of *12* judging by paper chromatography tests. The ether (*12*) exhibited the same absorption spectrum in visible light as *4*.

On hydrolysis in ether-methanol containing 5 % KOH *12* (0.1 mg) was 40 % converted into *4* after 3 h and was completely hydrolysed after 22 h.

Treatment with acid chloroform. 4 (0.3 mg) was treated with 0.03 N dry hydrogen chloride in ethanol-free chloroform for 10 min in daylight. An approximately 10 % conversion to a product with an absorption spectrum and paper-chromatographic properties indistinguishable from those of the lycopenal (*2*) was observed. Longer treatment resulted in undefined decomposition products. However, *4* proved considerably more resistant towards acid than did rhodopinol (*5*).

Dehydration to lycopenal (*2*). *4* (0.43 mg) in dry pyridine (5 ml) was treated with phosphorus oxychloride (0.05 ml) for 40 min at 50°C. After column chromatography the pigment recovery was 47 %. The recovered pigment comprised *2* (86 %) and undefined decomposition products. The product (*2*) could not be crystallized, exhibited an absorption spectrum in visible light superimposable on that of natural *2* and *4* (*cf.* Fig. 5), and had a partition ratio 98:2 in petroleum ether/95 % methanol. On iodine catalysis, natural and synthetic *2* gave rise to the same stereoisomeric set. The corresponding *trans*, neo A, and neo U isomers could not be separated by paper chromatography; *cf.* Table 3.

Hydride reduction to rhodopinol (*5*). *4* in dry ether was quantitatively reduced in a few minutes with lithium aluminium hydride to *5*; pigment yields were 75—90 %. *5* thus produced normally comprised *trans* (*ca.* 20 %, abs. max. (362), 450, 474, and 503 mμ, % D_B/D_{II} = 14, % III/II = 48 in acetone) and neo A (*ca.* 80 %, abs. max. 346, 363, (440), 467, and 495 mμ, % D_B/D_{II} = 44,½% III/II = 28 in acetone), *cf.* analogous spectra for *18* in Fig. 6. The product (*5*) had absorption spectra and adsorptive properties identical with those of natural *5*, as found by co-chromatography tests.

Ethyl rhodopinylidenacetate (*27*). To *4* (5.3 mg) in dry benzene (10 ml) *26* (40 mg) was added. The mixture was refluxed (4 h) after which paper chromatography revealed complete conversion to *27*. The pigments were transferred to benzene in the usual manner and submitted to column chromatography; pigment recovery was 81 %. *27* could not be crystallized. Absorption data in visible light are given in Table 2 and Fig. 7.

Rhodopinylidenacetic acid (*28*). *27* was completely saponified after 18 h when treated with 10 % KOH in methanol (4 ml). The acid was transferred to benzene on acidification of the aqueous hypophase with acetic acid; pigment recovery was 80 %. Absorption data for *28* are given in Table 2 and Fig. 7. On partition between petroleum ether and alkaline (KOH) methanol containing 30 % water, *28* was completely hypophasic. After acidification (acetic acid) of the hypophase, *28* was completely epiphasic.

2-Rhodopinylidene ethanol (*29*). *27* (2.5 mg) in dry ether was reduced with lithium aluminium hydride in the usual manner; pigment recovery was 81 %. The reaction mixture contained exclusively *29*, comprising three zones on paper chromatography, see Tables 2 and 3 and Fig. 7 for properties.

Table 3. Adsorptive properties observed for the new carotenals and their derivatives.

Carotenoid derivative	Required eluent from alumina activity grade 2	R_F-value on kieselguhr paper [a]				
		0 % [b]	2 %	5 %	10 %	20 %
1	25 % ether in petroleum ether	*0.54*	*0.86*			
2	50 % ether in petroleum ether		0.70, *0.58*, 0.34			
3	100 % ether or 100 % benzene			*0.31*, 0.33, 0.43, 0.55		
3 silyl ether		*0.42*				
4	100 % ether			0.22, *0.38*		
5	0.5—1.5 % methanol in petroleum ether				*0.24*, 0.27, 0.45	
5 disilyl ether			*0.90*			
6				*0.59*		
7	20 % ether in benzene					*0.50*
8	10 % methanol in ether				*0.21*, 0.32	
9	15 % acetone in petroleum ether		*0.63*	*0.34*		
10	15—25 % acetone in petroleum ether				*0.76*, 0.78, 0.85	
11			*0.43*			
12			*0.49*			
14					*0.57*	
16						*0*
17	50 % ether in petroleum ether			0.30, *0.69*		
18				*0.23*, 0.38		
19				*0.70*, 0.78		
20				*0.19*, 0.29		
21					*0.40*, 0.55, 0.68	
22	50 % ether in petroleum ether		*0.20*, 0.45, 0.56, 0.71			
23				0.30, *0.70*		
25				0.40, *0.52*		
27	20 % acetone in petroleum ether			*0.75*		
28						*0.14* [c]
30					*0.24*, 0.41, 0.50	

[a] R_F-values for observed stereoisomers are given. The stereoisomer absorbing light at longest wavelengths is indicated by italics.
[b] Acetone in petroleum ether.
[c] In 30 %.

Rhodopinylidenacetaldehyde 30. Attempts to oxidize *29* (2 mg) with *p*-chloranil in the standard manner resulted in decomposition products only. *30* was not obtained by treatment of *29* (0.12 mg) in benzene with air in the presence of catalytic amounts of iodine in light.[19]

Rhodopinol (*5*)

Crystallization could not be effected. After re-chromatography on alumina and precipitation twice from dry acetone-petroleum ether, a mauve-black precipitate was obtained on the glass wall in low yield; m.p. around 182°C. Solutions of paper-chromatographically pure *5* were used for experiments.

Absorption spectrum in visible light. Natural *5* was isolated predominantly (*ca.* 70 %) as a neo B isomer, abs. max. 345, 362, (440), 466, and 495 mμ, % D_B/D_{II} = 42, % III/II = 25 in acetone. The *trans* isomer had abs. max. at 362, 448, 472, and 503 mμ, % D_B/D_{II} = 16, % III/II = 48 in the same solvent. The spectrum corresponded to that of *18* in Fig. 6. A neo A isomer [4] was not readily separated from the *trans* isomer.

IR-spectrum. In a KBr pellet abs. max. were located at 3430 (OH); 2860 (CH); 1625; 1450 (CH_2); 1360 (CH_3); 1250; 1150 (tert. OH); 1025 (prim. allylic OH?); 962 (*trans* disubst. double bond); 905 (C(=C)–C–O), and 810 cm^{-1} (*trans* disubst. double bond); see Fig. 1. In chloroform solution the 1500–1700 cm^{-1} region was more complex.

NMR-spectrum. The spectrum in $CDCl_3$ at 60 Mc/sec had major signals at 3.30–4.10 (olefinic protons), 8.05 (in-chain methyl), 8.20 (end-of-chain methyl), 8.30–8.41 (isopropylidene) and 8.83 τ (*gem.* methyl at tert. oxygen substituent).

Partition tests. The values 12:88 in petroleum ether/95 % methanol and 61:39 in petroleum ether/85 % methanol have already been reported.[4]

Attempted dehydration of allylic hydroxyl. a) Standard treatments of *5* (0.1–0.2 mg) with 0.03 N HCl–$CHCl_3$ reagent for 15 min gave about 60 % pigment recovery generally. The recovered pigment consisted of yellow-orange undefined decomposition products only. b) Treatment of the tosylate (*16*, 0.3 mg) in ether (10 ml) with 10 % KOH-methanol (5 ml) for 5 min resulted in 30 % pigment recovery and no formation of elimination products with prolonged chromophores.

Oxidation to rhodopinal (4). a) [19] To *5* (50 μg) in benzene (5 ml) iodine (2.5 μg) in petroleum ether (0.5 ml) was added. The mixture was shaken in air in daylight. After 4 h *4* represented 10 % of the reaction mixture. b) [21] To *5* (30 μg) in carbon tetrachloride (5 ml) a spatula tip of silver oxide was added. After 3 h a pigment recovery of 40 % was obtained. The reaction mixture contained *4* (20 %). c) Oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone [20] quickly destroyed all carotenoid. With *p*-chloranil a certain formation of *4*, but very low pigment recovery, was recorded after 24 h.

Rhodopinyl bis-trimethylsilyl ether. This ether was prepared from *5* (0.37 mg) as described for *12* above.

Rhodopinyl acetate (9). *5* (2.04 mg) in pyridine (2 ml) was treated with acetic anhydride (0.2 ml) for 5 h; pigment recovery was 100 %. The reaction mixture contained unreacted *5* (18 %) and *9* (82 %). *9* was isolated mainly as a neo A isomer with abs. max. 345, 362, (445), 466, and 493 mμ in acetone, which constituted 80 % of the iodine catalyzed equilibrium mixture. The absorption spectrum of the *trans* isomer corresponded to that of *5*. *9* exibited a partition ratio of 52:48 in petroleum ether/95 % methanol.

9 (0.22 mg) was saponified in 5 % KOH-methanol for 2 h. The recovered carotenoid (98 %) consisted of *5* only.

Rhodopinyl tosylate (16). To *5* (13.4 mg) in dry pyridine (10 ml), *p*-toluenesulphonyl chloride (100 mg) was added. After 18 h a complete conversion to *16* was observed by paper chromatography. The carotenoid was transferred to benzene in the usual manner; pigment recovery was 69 %.

Hydride reduction of rhodopinyl tosylate (16) to rhodopin (13). To *16* (4.6 mg) in dry tetrahydrofuran (1 ml) and dry ether (4 ml) an excess of lithium aluminium hydride was added. The reaction was interrupted after 20 min, when a *ca.* 10 % conversion to *3* was observed. The products were isolated in the usual manner and subjected to column

chromatography. *3*, eluted with benzene, yield 0.6 mg (17 %), abs. max. (*trans*) 345, 362, 447, 472, and 506 mμ, was not obtained in the crystalline state. The iodine catalyzed equilibrium mixture contained *trans* (*ca.* 50 %), neo a (*ca.* 5 %), neo b (*ca.* 20 %), and neo c (*ca.* 25 %). In co-chromatography tests with the corresponding stereomutation mixture of authentic *3*, no separation of the corresponding stereoisomers was achieved.

The reduction product (*3*, 0.15 mg) was silylated as described for *12* above in a parallel experiment with authentic *3*. The corresponding trimethylsilyl ethers were readily formed and could not be separated by co-chromatography.

3,4,3′,4′-Tetrahydro-spirilloxanthinal (*17*)

Crystallization. *17*, chromatographed three times on alumina, gave no crystals from a series of solvent pairs. Solutions, paper-chromatographically free from other carotenoids, were used for experiments. In total, *ca.* 3 mg was available.

Absorption spectrum in visible light. The spectrum of the naturally occurring stereoisomer was superimposable on that of *2* and *4*, see Fig. 5.

IR-spectrum. The spectrum recorded in KBr (partly reproduced in Fig. 4) had abs. max. at 3400 (water and overtone carbonyl); 2840 (CH); 1680—60 (conj. aldehyde); 1600; 1460 (CH_2); 1385—60 (CH_3); 1265; 1200; 1160; 1110; 1080 (OCH_3); 1015; 968 (*trans* disubst. double bonds); 905 and 830 cm^{-1}.

Partition test. *17* had a partition ratio of 63:37 in petroleum ether/95 % methanol.

Stereoisomerisation. The iodine catalyzed equilibrium mixture contained traces only of a neo U isomer besides the natural isomer.

Acetylation. *17* (60 μg) in dry pyridine (1 ml) was treated with acetic anhydride (0.6 ml) for 20 h; pigment recovery was 83 %. The reaction mixture contained unreacted *17* only.

3,4,3′,4′-Tetrahydro-spirilloxanthinol (*18*). *17* (0.15 mg) in dry ether was readily reduced with lithium aluminium hydride in the usual manner; pigment recovery was 81 %. The reaction mixture contained *18* only, present as two stereoisomers, neo A (95 %) with abs. max. (349), 361, (441), 466, and 493 mμ and *trans* (5 %) with abs. max. (361), 450, 473, and 505 mμ in acetone, see Fig. 6.

3,4,3′,4′-Tetrahydro-spirilloxanthinyl acetate (*19*). *18* (73 μg) in dry pyridine (1.5 ml) was treated with acetic anhydride (0.3 ml). The acetylation was completed in 25 min, and no intermediary acetates were observed during the reaction period. *19* thus formed comprised the *trans* isomer (10 %) and a neo A isomer (90 %).

19 (47 μg) was hydrolyzed with 5 % KOH-methanol (10 ml) for 1 1/2 h; pigment recovery was 85 %. The reaction mixture contained *18* only.

Oxidation of 18 to 3,4,3′,4′-tetrahydro-spirilloxanthinal (*17*). *18* (40 μg) in benzene (1 ml) was treated with *p*-chloranil (0.15 mg) and iodine (10 μg) in petroleum ether (1 ml) for 16 h in sodium light. A 5 % conversion to *17* was observed paper-chromatographically. Nickel peroxide (5 mg) was added. After 3 h stirring the amount of *17* had increased to 15 % of total. The products were isolated in the usual manner; pigment recovery was 45 %.

3,4,3′,4′-Tetrahydro-spirilloxanthinaldoxime (*20*). To *17* (2.22 mg) in dry benzene (3 ml) and dry pyridine (1.5 ml) hydroxylamine hydrochloride (10.9 mg) was added. After 35 min at 50°C a quantitative conversion to *20* was observed. The pigment was transferred to petroleum ether in the usual manner; pigment recovery was 95 %.

In a parallel experiment β-apo-2′-carotenal (C_{37}) oxime was prepared in the same way from the corresponding carotenal (2.22 mg). After 35 min at 50°C complete conversion to the oxime was observed; pigment recovery was 100 %. The *trans* oxime had $R_F = 0.58$ on kieselguhr paper (5 % acetone in petroleum ether) compared with $R_F = 0.30$ (2 % acetone in petroleum ether) for the carotenal, and had abs. max. at (460), 487, and 520 mμ in acetone.

3,4,3′,4′-Tetrahydro-spirilloxanthinal 2,4-dinitrophenylhydrazone (*21*). To *17* (0.7 mg) in glacial acetic acid (2 ml) 2,4-dinitrophenylhydrazine (3 mg) was added. After 30 min at 100°C a complete conversion to *21* was observed. The product was transferred to benzene in the usual manner; pigment recovery was 97 %. *21*, crystallized from ether-petroleum ether, exhibited abs. max. at 516 mμ in acetone, see Fig. 6. The IR-spectrum in a KBr pellet had abs. max. at 3450, 2900, 1615, 1595, 1510, 1420, 1380, 1355, 1310, 1260, 1210,

1130; 1080 (OCH_3), 1052, 962 (*trans* disubstituted double bonds), 920, 825, and 740 cm^{-1}, see Fig. 4, also for the spectrum of 2,4-dinitrophenylhydrazine. The mass spectrum showed a molecular ion at *m/e* 794 as calculated for *21*.

In a parallel experiment β-apo-2′-carotenal (C_{37}) 2,4-dinitrophenylhydrazone was prepared in the same way from the corresponding carotenal (0.7 mg). After 30 min at 100°C a quantitative formation of the hydrazone was observed; pigment recovery was 97 %. The crystalline *trans* hydrazone (from ether-petroleum ether) had an $R_F = 0.40$ on kieselguhr paper (10 % acetone in petroleum ether), abs. max. 516 mμ in acetone, and IR-absorption (KBr pellet) at 3450, 2820, 1610, 1590, 1505, 1420, 1390, 1360, 1335, 1305, 1265, 1215, 1160, 1120, 1080 (weak compared with the corresponding band in *21*), 1052, 1000, 962, 915, 830, 742, and 722 cm^{-1}.

3,4,3′,4′-Tetrahydro-spirilloxanthin (*22*).[4] Adsorptive properties are cited in Table 3.

3,4,3′,4′-Tetrahydro-spirilloxanthinal thioketal (*23*). The procedure of Hauptmann [34] was followed. To *17* (0.8 mg) in dithioglycol (0.6 ml) anhydrous sodium sulphate (500 mg) was added. No change in pigment composition was observed after 5 h stirring. Zinc chloride (50 mg) was added, which destroyed all carotenoids in 10 min.

In a parallel experiment β-apo-2′-carotenal (C_{37}) was treated in the same way, omitting the zinc chloride. After 2 h the presumed thioketal comprised 30 % of the reaction mixture. The unreacted carotenal and the more orange product (2 zones) had R_F-values of 0.19, 0.39, and 0.60, respectively, on kieselguhr paper (1% acetone in petroleum ether).

3,4,3′,4′-Tetrahydro-spirilloxanthinaldoxime acetate (*24*). To the oxime (*20*, 0.16 mg) in dry ether (0.7 ml) acetic anhydride (1 ml) was added. After 25 min at 50°C paper chromatography revealed quantitative conversion to *24*. The product was transferred to ether in the usual manner; pigment recovery was 83 %. *24* comprised two zones (Table 3), the major ($R_F = 0.70$) isomer had abs. max. 362 and 485 mμ in acetone (see Fig. 6).

In a parallel experiment β-apo-2′-carotenal(C_{37}) oxime (2.2 mg) was treated in the same way. After 30 min at 100°C complete conversion to the corresponding acetate was observed; pigment recovery was 89 %. The *trans* oxime acetate had an $R_F = 0.63$ (5 % acetone in petroleum ether) and abs. max. at 383, 490.5, and 520 mμ in actone.

Alkali treatment of 3,4,3′,4′-tetrahydro-spirilloxanthinaldoxime acetate (*24*). *24* (0.14 mg) in ether (5 ml) was treated with 10 % KOH-methanol (10 ml) for 10 min. Complete conversion to the aldoxime (*20*) was then observed; pigment recovery was 98 %. The product (*20*) had a partition ratio in petroleum ether/95 % methanol of 36:64 (neutral or acidic hypophase) and 27:73 (alkaline hypophase) and in petroleum ether/85 % methanol 88:12 (neutral or acidic hypophase) and 59:41 (alkaline hypophase). The product (*20*, two isomers) had R_F-values of 0.32 and 0.41 on aluminium oxide paper (20 % acetone in petroleum ether).

In a parallel experiment β-apo-2′-carotenal (C_{37}) oxime acetate (2.0 mg) was treated in the same way for 1 h; pigment recovery was 83 %. Quantitative hydrolysis to the corresponding oxime was then achieved. The product had a partition ratio in petroleum ether/95 % methanol of 59:41 (neutral or acidic hypophase) and 40:60 (alkaline hypophase).

3,4,3′,4′-Tetrahydro-spirilloxanthinaldoxime methyl ether (*25*). To *20* (0.13 mg, obtained by saponification of *24*) in benzene (3 drops) ether (5 ml) saturated with diazomethane was added. After 17 h at −20°C complete conversion to *25* was observed; pigment recovery was 86 %. *25* comprised two isomers (Table 3). The main isomer (70 % of total, $R_F = 0.52$) had abs. max. at 362 and 487 mμ in acetone (see Fig. 6).

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