Phytochemistry, 1976, Vol. 15, pp. 1749–1751. Pergamon Press. Printed in England.

CROSS-CONJUGATED CAROTENOID ALDEHYDES IN *RHODOPSEUDOMONAS VIRIDIS*

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(*Received* 15 *March* 1976)

Key Word Index—*Rhodopseudomonas viridis*; Rhodospirillaceae; cross-conjugated carotenals; 13-*cis*-ψ,ψ-caroten-20-al; 3,4-didehydro-1,2-dihydro-ψ,ψ-caroten-20-(or 20′)-al; acetonyl derivatives.

Abstract—Two carotenoid aldehydes isolated from *Rhodopseudomonas viridis* (Rhodospirillaceae) have been identified as lycopen-20-al (13-*cis*-ψ,ψ-caroten-20-al) and the novel 3,4-didehydro-1,2-dihydrolycopen-20 (or 20′)-al (3,4-didehydro-1,2-dihydro-ψ,ψ-caroten-20-al or 3′,4′-didehydro-1′,2′-dihydro-ψ,ψ-caroten-20-al). Some extracts also contained acetonyl derivatives, probably artefacts produced from the cross-conjugated carotenoid aldehydes and acetone under alkaline conditions. The distribution of cross-conjugated caroten-20-als in photosynthetic bacteria is discussed.

INTRODUCTION

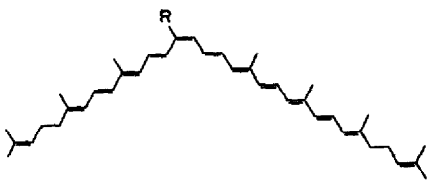
The photosynthetic bacterium *Rhodopseudomonas viridis* (Rhodospirillaceae) accumulates large amounts of carotenoid hydrocarbons. The main constituents are neurosporene (7, 8-dihydro-ψ,ψ-carotene), lycopene (ψ,ψ-carotene) and a novel series of 1,2-dihydrocarotenoids, including 1,2-dihydroneurosporene (1,2,7,8-tetrahydro-ψ,ψ-carotene), 1,2-dihydrolycopene (1,2-dihydro-ψ,ψ-carotene) and 1,2-dihydro-3,4-didehydrolycopene (3,4-didehydro-1,2-dihydro-ψ,ψ-carotene) [1,2]. Examination of extracts of large scale (30 litres) cultures of *R. viridis* revealed the presence of a considerable number of more polar carotenoids mostly present in very small amounts. Notable among these was a group of strikingly purple-coloured compounds with similar chromatographic properties. These compounds have been isolated and their structures investigated.

RESULTS AND DISCUSSION

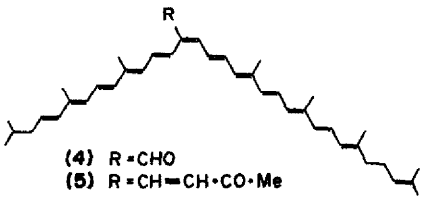
From the extract of *R. viridis* a group of purple compounds was obtained by column chromatography and separated by TLC into four components. Each compound exhibited a 'rounded' absorption spectrum but $NaBH_4$ reduction restored the typical carotenoid three-peak fine structure and caused a bathochromic shift of about 35 nm. This behaviour is characteristic of carotenoids containing a carbonyl group in conjugation with the main polyene chromophore. A very strong '*cis*-peak' (strong absorption in the 360–375 nm region) was observed in the absorption spectrum in every case. The general features of the spectra were reminiscent of those reported by Liaaen-Jensen and co-workers [3–6] for the unusual cross-conjugated aldehydes lycopen-20-al (13-*cis*-ψ,ψ-caroten-20-al) and rhodopin-20 (or 20′)-al (13-*cis*-1-hydroxy-1,2-dihydro-ψ,ψ-caroten-20-al or 13-*cis*-1′-hydroxy-1′,2′-dihydro-ψ,ψ-caroten-20-al). The *R. viridis* compounds were characterized as detailed below.

Lycopen-20-al (13-cis-ψ,ψ-*caroten-20-al*) (**1**).

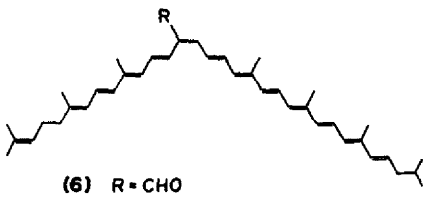
This compound had λ_{max} (EtOH) at 372 and approx 492 nm, shifted to 371, 444, 467 and 496 nm by $NaBH_4$ reduction. This suggested a *cis*-lycopene chromophore, with a carbonyl group in conjugation in the natural com-



(1) R = CHO
(2) R = CH=CH–COMe
(3) R = CH=CH–CHOH–Me



(4) R = CHO
(5) R = CH=CH·CO·Me



(6) R = CHO
(7) R = CH=CH·CO·Me

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pound. The MS had the parent ion M^+ at *m/e* 550 (100%, $C_{40}H_{54}O$) showing that the carbonyl group was the only oxygen function present. A major fragment ion at *m/e* 481 (14%, M-69, *m** 421; $481^2/550 = 420.6$) showed the presence of at least one acyclic (lycopene) end-group. In addition to a fragment ion at *m/e* 458 (8%, M-92, *m** 381; $458^2/550 = 381.4$) due to loss of toluene by typical carotenoid in-chain fragmentation, major fragment ions were present at *m/e* 444 (42%, M-106, *m** 358; $444^2/550 = 358.4$) and 430 (25%, M-120) due to losses of benzaldehyde and *m*-tolualdehyde respectively. The loss of these fragments indicates the presence of an aldehyde group in place of one of the in-chain methyl groups [6]. Similarly the MS of the $NaBH_4$ reduction product had M^+ at *m/e* 552 ($C_{40}H_{56}O$) and major fragment ions at *m/e* 444 and 430 due to losses of 108 (benzyl alcohol) and 122 (3-methyl benzyl alcohol) mass units, confirming the reduction of the in-chain aldehyde group to an alcohol.

Although the available data do not permit the unequivocal assignment of the aldehyde group to the C-20 rather than the C-19 position, the similarities in spectroscopic properties (mass and absorption) strongly suggest that the *R. viridis* compound is identical to lycopen-20-al first isolated from *Chromatium warmingii* [3], and characterized and synthesized by Liaaen-Jensen and coworkers [5–7]. The similarity of the absorption spectra, especially in the *cis*-peak region indicates that the lycopen-20-al from *R. viridis* also has the 13-*cis* configuration.

3,4-Didehydro-1,2-dihydrolycopen-20 (20′)-al [*3,4-didehydro-1,2-dihydro-ψ,ψ-caroten-20-al* (**4**) *or 3′,4′-didehydro-1′,2′-dihydro-1′,2′-dihydro-ψ,ψ-caroten-20-al* (**6**).

The general appearance of the spectrum of the main compound in the group was similar to that of lycopen-20-al but had λ_{max} (EtOH) at 376, 505 nm. $NaBH_4$ reduction caused a shift to 358, 372, 450, 475, 506 nm and restoration of the normal carotenoid three-peaked fine structure, indicating that the natural compound contained a carbonyl group in conjugation with a dodecaene chromophore like that of 3,4-didehydro-1,2-dihydrolycopene (3,4-didehydro-1,2-dihydro-ψ,ψ-carotene) also found in *R. viridis*. The MS showed that the compound was isomeric with lycopen-20-al, with M^+ at *m/e* 550 (100%, $C_{40}H_{54}O$) and a fragment ion at *m/e* 481 (7%, M-69, *m** 421; $481^2/550 = 420.7$) showing the presence of a lycopene end-group. As with lycopen-20-al, fragment ions at *m/e* 444 (16%, M-106, *m** 358; $444^2/550 = 358.4$) and 430 (10%, M-120) due to losses of benzaldehyde and *m*-tolualdehyde were present in addition to the usual M-92 (toluene) and M-106 (xylene) peaks at *m/e* 458 and 444 respectively, and an intense fragment ion at *m/e* 378 (38%, *m** 260, $378^2/550 = 259.8$) due to loss of 172 m.u. largely replaced the M-158 peak. The $NaBH_4$ reduction product had M^+ at *m/e* 552 (60%, $C_{40}H_{56}O$) and fragment ions at *m/e* 444 (6%, *m** 357, $444^2/552 = 357.1$), 430 (11%) and 378 (22%, *m** 259; $378^2/552 = 258.8$) due to losses of 108, 122 and 174 m.u. The MS data thus confirmed the presence of an aldehyde group at C-19, 20, 19′, or 20′ as in the lycopenal.

The amount of material available was small, and the compound appeared to be unusually labile (the instability of lycopen-20-al and rhodopin-20(20′)-al has been noted previously [5]), so that several attempts to obtain a NMR spectrum that could be analyzed fully were unsuccessful. However the presence of the aldehyde group was confirmed by a poorly defined absorption at δ 9.5, and a characteristic doublet at 0.89 (J 6 Hz) proved the presence of the 1,2-dihydro end group.

The general similarity of the absorption spectrum, especially the *cis*-peak region, to that of lycopen-20-al, indicates a probable 13-*cis* configuration with the aldehyde group at C-20 (or C-20′), although the alternative C-19 (or C-19′) position is not definitely eliminated. It is not possible by conventional spectroscopic methods to distinguish between the two alternative structures 3,4-didehydro-1,2-dihydro-ψ,ψ-caroten-20-al (**4**) and 3′,4′-didehydro-1′,2′-dihydro-ψ,ψ-caroten-20-al (**6**). Extensive and difficult degradation would probably be necessary, and this is not practicable with the small samples thus far available.

20-Acetonyllycopenal (**2**) and *20(20′)-acetonyl-3,4-didehydro-1,2-dihydrolycopenal* (**5** or **7**).

The remaining two compounds were not always present in extracts of *R. viridis*, but were found mainly when the cells had been extracted with acetone and the extracts saponified. The absorption spectra showed that these were also carbonyl compounds. This was confirmed by $NaBH_4$ reduction, although this took place more slowly than with the aldehydes described above. The MS showed that both were C_{43} compounds and the available data were in agreement with their being acetonyl derivatives of lycopen-20-al and 3,4-didehydro-1,2-dihydrolycopen-20-al, produced as artefacts by aldol condensation between these aldehydes and traces of acetone present during saponification. Similar products have been characterized by Schmidt *et al.* [8] in their studies of the carotenoids of some strains of *Rhodopseudomonas acidophila*.

Compound **2** had λ_{max} (EtOH) at 374 and 488 nm, shifting to 370, 443, 469 and 498 nm on $NaBH_4$ reduction, suggesting the presence of a carbonyl function in conjugation with a *cis*-lycopene chromophore. The MS had the parent ion M^+ at *m/e* 590 (8%, $C_{43}H_{58}O$) and a fragment ion at *m/e* 521 (2.5%, M-69, *m** 460; $521^2/590 = 460.0$) due to 'bis-allylic' cleavage of the C-3,4 bond of the lycopene end-group. Other major fragment ions were present at *m/e* 547 (1%,*m** 507; $547^2/590 = 507.1$), 444 (0.5%) 430 (4%) and 378 (3%, *m** 242; $378^2/590 = 242.2$), due to losses of 43, 146, 160 and 212 m.u., characteristic fragmentations of acetonyl derivatives of caroten-20-als [8]. The MS was virtually identical to that of 20-acetonyllycopenal published by Schmidt *et al.* [8]. The MS of the $NaBH_4$ reduction product had the parent ion M^+ at *m/e* 592, and major fragment ions at *m/e* 444, 430 and 378 due to losses of 148, 162, and 214 m.u., thus confirming the location of the acetonyl group on a side-chain methyl substituent. It therefore has structure **2**, (20-acetonyllycopenal) and the $NaBH_4$ reduction product is **3**.

Compound **5** had λ_{max} (EtOH) at 378 and 506 nm, shifting to 360, 374, 452, 477 and 507 nm on $NaBH_4$ reduction, indicating a carbonyl group in conjugation with a dodecaene chromophore. The MS (M^+ at *m/e* 590, $C_{43}H_{58}O$) was rather similar to that of 20-acetonyllycopenal, with major fragment ions at *m/e* 547, 444, 430 and 378 due to losses of 43, 146, 160 and 212 m.u. again proving the acetonyl substituent to be located on a side-chain methyl group (C-19 or C-20), but the relative inten-

sity of the fragment ion at *m/e* 521 (M-69) was substantially lower, suggesting the presence of only one lycopene end group rather than two as in 20-acetonyllycopenal. A similar situation was also observed in the mass spectra of **1** and **4** (or **6**) discussed above. A feature of the NMR spectrum of compound **5** was the presence of a six-proton doublet at δ 0.90 (J 6 Hz) proving the presence of a 1,2-dihydro end group. Compound **5** is thus the acetonyl derivative of 3,4-didehydro-1,2-dihydrolycopen-20 (or 20′) al, but again it is not possible to distinguish between the two alternative structures **5** and **7**.

The formation of **2** and **5** (or **7**) by treatment of lycopen-20-al and 3,4-didehydro-1,2-dihydrolycopen-20 (or 20′)-al with traces of acetone under basic conditions was demonstrated, thus confirming the relationship between the acetonyl derivatives and the caroten-20-als.

3,4-Didehydro-1,2-dihydrolycopen-20 (or 20′)-al is the first carotenoid to be reported that contains both the cross-conjugated aldehyde system and the 1,2-dihydro end group characteristic of the *R. viridis* carotenoid hydrocarbons. Several cross-conjugated carotenals (lycopen-20-al, rhodopin-20-al and its glucoside, and some methoxycarotenals) have previously been found in extracts of other photosynthetic bacteria. The distribution of these compounds has been summarized by Schmidt [9]. One interesting feature is that their occurrence seems to be limited to strictly anaerobic organisms.

Possible biosynthetic schemes have been proposed [6] for formation of the caroten-20-als, but no supporting evidence is yet available. It is suggested that the caroten-20-ols may be intermediates in the formation of the aldehydes, since rhodopin-20-ol is present in most extracts that contain rhodopin-20-al. Although no such compounds have yet been detected in *R. viridis*, extracts of this organism do contain a considerable number of polar carotenoids, all present in very small amounts, and it is possible that future work may reveal the presence of carotenols related to the cross-conjugated aldehydes described in this paper.

EXPERIMENTAL

Organism and culture conditions. Cultures of *Rhodopseudomonas viridis* were obtained as kind gifts from Dr. K. E. Eimhjellen, Trondheim, Norway, Dr. Karin Schmidt, Göttingen, West Germany, and Dr. E. Hilary Evans of this Department. The organism was cultured anaerobically in the light as previously described [2].

Pigment extraction and purification. Cells were harvested by centrifugation and the pigments were extracted with MeOH and Me_2CO, or in some cases MeOH alone, and saponified according to standard procedures [10]. The saponified extracts were chromatographed on columns of neutral alumina (Brockmann grade III) [10], and after elution of the carotenoid hydrocarbons with 1% Et_2O in petrol, a second fraction, eluted with 20% Et_2O in petrol, was collected. TLC of this fraction on Si gel G with 15% Et_2O in petrol gave four purple bands with R_f 0.6, 0.5, 0.35, 0.25 respectively. In those extracts prepared without the use of Me_2CO the two lowest R_f bands were absent. Bands of R_f 0.6 and 0.25 were each further purified by successive TLC on MgO–Kieselguhr G (Me_2CO–C_6H_6, 1:1) and Si gel G (20% Et_2O in petrol) to give lycopen-20-al (**1**), 3,4-didehydro-1,2-dihydrolycopen-20(20′)-al (**4**), 20-acetonyllycopenal (**2**) and 20(20′)-acetonyl-3,4-didehydro-1,2-dihydrolycopenal (**5**) respectively. Details of the absorption spectra, MS and NMR spectra of these compounds are given in the Results and Discussion section. $NaBH_4$ reduction was performed by standard methods [10] and the products were purified by TLC on Si gel G, with Et_2O–petrol (1:1) as developing solvent. Absorption spectra were recorded in EtOH. NMR spectra (100 mHz) were kindly determined by Dr. G. Englert, F. Hoffmann-La Roche and Co. Ltd., Basle, Switzerland, and by P.C.M.U., Harwell, England. MS (at 12 eV or 70 eV) were recorded by Mrs. A. M. Ball or Mr. J. R. Ireland. The direct inlet system was used with an ion source temperature of 200–220°.

Acknowledgements—We thank the Science Research Council for financial support. A.B.-A. was in receipt of a research fellowship from the Hebrew University of Jerusalem and a Johnston Fellowship in Biochemistry from the University of Liverpool. R.K.S. thanks the British Council for financial assistance, and H.C.M. thanks the Wellcome Trust for a travel grant. We are grateful to Mrs. S. K. Pigott and Mrs. M. K. Anderson for technical assistance.

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