Eur Biophys J (1988) 16:193–205

European Biophysics Journal

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The polarized UV-absorption spectra and the crystal structure of two different monoclinic crystal forms of the retinal homologue β-8′-apocarotenal

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Received February 11, 1988/Accepted June 27, 1988

Abstract. During the visual process, light absorption in the 11-*cis* retinylidene chromophore leads to a rapid *cis-trans*-isomerization which initiates the phototransduction step. Important spectroscopic properties of this chromophore can be derived from polarized UV-absorption spectra of crystalline 11-*cis*-retinal if a parallel X-ray structure analysis is performed. Several questions about the relation between molecular geometry and spectroscopic behavior could not be answered from these spectra. All crystal forms of 11-*cis*-retinal contain this molecule in its 6-s-*cis*-ring conformation. For the retinal homologue, β-8′-apocarotenal (APC), however, two crystal forms with different ring conformation can be grown. The spectrum of α-APC (6-s-*cis*) shows a vibronic structure whereas that of β-APC (6-s-*trans*) is diffuse but has a distinct shoulder on the low energy side of the main band. This S-band is typical for retinal spectra and has been ascribed to a transition into a $^1A_g^{-*}$-state. The appearance of the S-band is not correlated with a 6-s-*cis*-conformation as suggested by the retinal spectra but is due to intermolecular interactions: β-APC has a dense dimer packing and a strong electrostatic interaction between the π-electron systems. This might cause the "forbidden" $^1A_g^{-*}$-transition. On the other hand, this interaction is missing in the loose and polar packing of α-APC which favors vibration in the polyene chain. This finding is remarkable in view of the photodynamic behavior of the visual chromophore for which strong electrostatic interactions with the protein helices of its binding site have to be postulated.

Key words: Apocarotenal, retinal homologue, visual chromophore, polarization absorption spectra, spectroscopic properties

Introduction

The 11-*cis*-isomer of retinal, the aldehyde of vitamin-A, forms the light absorbing chromophore of the great majority of all known visual pigments in the animal kingdom (Wald 1968). After light absorption in the photopigment the molecule is photoisomerized within a few picoseconds into an all-*trans* like configuration which initiates the phototransduction process (Applebury 1980). Therefore, the absorption behavior and the excited state dynamics of this molecule have been intensively investigated (Ottolenghi 1980; Birge 1981). In this connection two questions are of special interest: *1.* The appearance of a new electronic absorption band on the low energy side of the one-electron-absorption as well as in the two-photon excitation spectra (Birge et al. 1982a; Drikos et al. 1984a, b; Drikos and Rüppel 1984), and *2.* the failure of all attempts to resolve a vibrational structure in the main absorption band of all-*trans* retinal in Shpolskij-matrices and in mono-crystals (Hudson and Loda 1981; Birge et al. 1982a, b).

The investigation of this flexible molecule has been especially favorable in the crystalline state. In the crystal the conformation of the molecule and its environment is fixed. This can be well established by X-ray structure analysis.

The UV-polarization absorption spectra of all-*trans* retinal (ATR), which in the organic crystal has a twisted 6-s-*cis* conformation, shows a structureless main band with a shoulder on its low energy side

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Abbreviations: APC: 8′-β-Apocarotenal; α-APC/β-APC: α/β-form of crystallized APC; α-*CIS*/β-*CIS*: α/β-form of crystallized 11-*cis*-retinal; ATR: all-*trans* retinal; UV: ultraviolet light; CI: quantum-mechanical calculation employing *c*onfiguration *i*nteraction; PPP-MRD: quantum-mechanical calculations after *P*ariser, *P*arr, *P*ople employing *m*ulti*r*eference *d*eterminants; S-bands: shoulder on main absorption band; R, S: right, left enantiomer; EtOH: ethyl alcohol; PE: petroleum ether; E: direction of electric vector of incident light; *b*: crystallographic *b*-axis.

(Drikos et al. 1984b; Drikos and Rüppel 1984). Similar behavior is shown by the crystal spectra of the isomer 6-s-*cis*, 11-*cis*, 12-s-*cis* retinal which forms the chromophore of the visual pigment and is thus of great biological interest (Drikos et al. 1984b).

For studying the absorption behavior of retinal homologues with an increasing number of conjugated double-bonds, β-8′-Apocarotenal (APC) (which is commercially available), was chosen. This retinal homologue has four additional i.e. a total of ten conjugated double bonds. From this compound two different crystal forms could be grown: the first one, which has already been described in the chemical literature, has a melting point of 138.5 °C (α-APC) (Karrer and Solmsen 1937). The other one, which is reported for the first time here shows a lower melting point at 132.3 °C (β-APC). Polarization-UV absorption spectra of both crystal forms were measured. The crystal spectrum of α-APC at 300 K has a main band with vibrational structure but without a low energy satellite band. The crystal spectrum of β-APC, however, shows a structureless main band with a distinct shoulder on its low energy side. To understand this particular spectral behavior an X-ray structure analysis of both crystal forms was performed. The polarization UV-absorption spectra of both forms will be discussed in terms of the results of this structure analysis.

Experimental

β-8′-Apocarotenal from FLUKA, Neu-Ulm, W.-Germany, was two times recrystallized from methanol. The dichroitic crystal platelets thus obtained, show a melting point at 138.5 °C (FP 1/2 Mettler, Gießen, W.-Germany) which agrees well with the value given in the literature for crystals of β-8′-apocarotenal (i.e. α-APC) (Karrer and Solmsen 1937). In the polarization microscope the α-APC crystal shows straight extinction.

By recrystallizing APC three times from petroleum ether very thin and extremely strong dichroitic crystal platelets are obtained (β-APC) which have a melting point at 132.3 °C. This crystal form also shows straight extinction in the polarization microscope.

Table 1. Cell parameters and physical data

	α-APC	β-APC
Molecular formula	$C_{30}H_{40}O$	$C_{30}H_{40}O$
Molecular weight/ g/mol	416.65	416.65
Habit	elongated parallel-epiped on (001)	elongated parallel-epiped on (001)
Melting point/°C	138.5(5)	132.3(3)
Space group	$P2_1$	$P2_1/a$
Number of molecules in elementary cell	2	4
Axis: a/Å	8.021(1)	17.725(4)
b/Å	9.458(3)	8.643(2)
c/Å	17.912(4)	18.317(4)
Angle/degree	95.15(1)	110.06(2)
Volume/Å^3	1,353.4	2,635.7
Density/g/cm^3 [calculated]	1.022	1.050

In order to measure polarization-UV absorption spectra, microcrystals which have a very small thickness of less than 0.1 μm must be used. These crystal platelets were grown on quartz slides from a solution of alkanes of different boiling points reaching saturation by quick evaporation of the lower boiling component (Drikos et al. 1984a). Increasing the crystallization temperature up to 80 °C enlarged the yield of thin microcrystals as compared with the procedure described before (Drikos et al. 1984a). The measurement of polarization-UV absorption spectra was performed by means of a microscope-spectrophotometer UMSP I (Zeiss Oberkochen, W.-Germany) which had additionally been equipped with a Zeiss double monochromator MM 12, a UV-glan prism and a metal grid of 25% transmission within the reference beam. Its data output was connected to an APPLE II plus computer (Apple Computer Inc. Cupertino, CAL, USA). The measuring method has been described by Drikos et al. 1984a.

For the X-ray structure determinations, crystals of α and β-APC were sealed in evacuated glass capillaries and protected from light. The measurements were carried out at 17 °C on a CAD-4 diffractometer (ENRAF-NONIUS, Delft, Netherlands) with graphite monochromated Mo-K_α radiation. Structure factors comprising symmetry equivalent reflections were evaluated from reflection profiles ($\omega/2\theta$ scans). Averaging over multiple measurements and equivalent reflections yielded unique sets of structure factors F_0^2 for α- and β-APC, respectively. For a comparison all residuals $R(F^2)$ listed in Table 1 are based on structure factors stronger than 2σ.

The structures were solved by direct method with the program MULTAN-76 (Main et al. 1976) and refined by full matrix least squares methods with the program system X-RAY-76 (Stewart et al. 1976) using weights $1/\sigma^2$ (F_0^2) and treating the structure factors weaker than 2σ as unobserved. The non-hydrogen atoms were refined anisotropically, the hydrogen atoms with fixed calculated thermal parameters. Table 6 in the Appendix gives the cell parameters, other physical crystal data, the final $R(F^2)$ values and the biggest minima and maxima of the final difference maps.

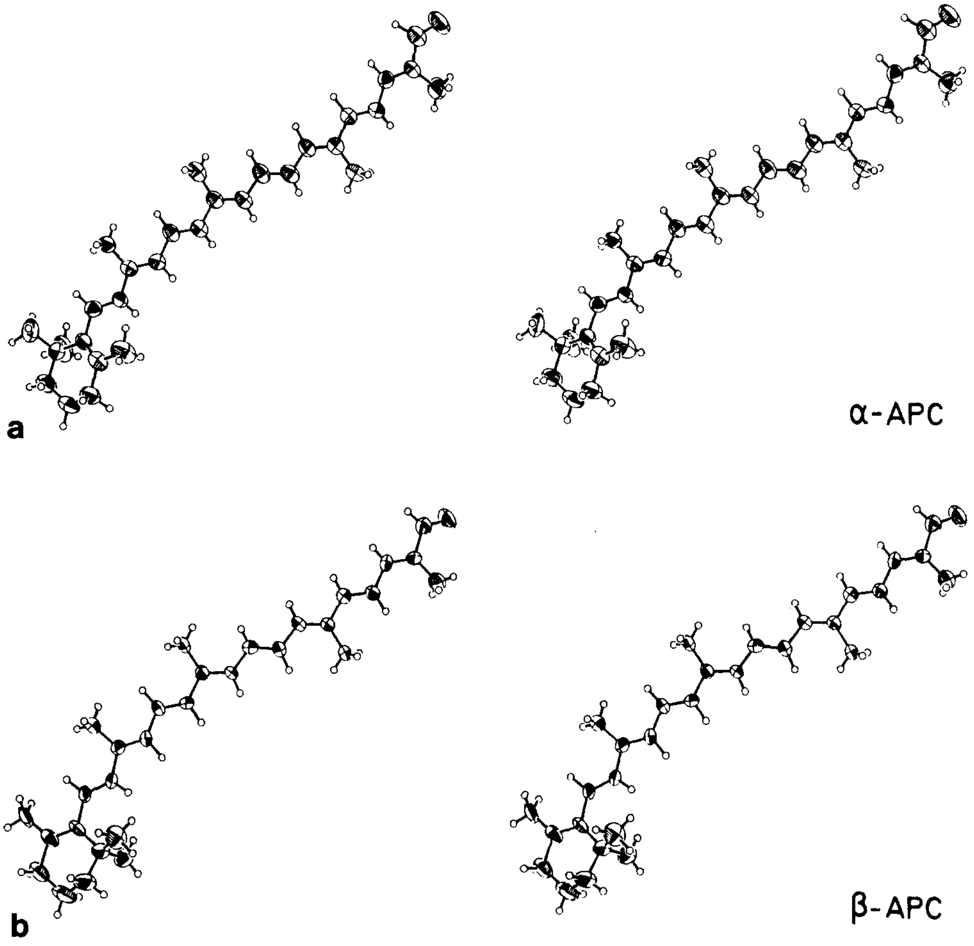

Fig. 1 a and b. Stereo picture of β-8′-apocarotenal molecule as found in α-APC (**a**) and β-APC (**b**) crystal form by X-ray structure analysis. 50% probability ellipsoids for non-hydrogen atoms. (ORTEP-plot Johnson 1976)

The structure of β-APC is based on measurements within a limited θ range only. Therefore, the estimated standard deviations for its parameters are almost ten times greater than those of the parameters of α-APC (c.f. Appendix, Table 6).

The cell parameters and other physical data of the α- and β-APC crystal forms are listed in Table 1.

Results and discussion

Structure analysis

The molecular structures of β-8′-apocarotenal (APC) in both its crystal forms, α- and β-APC, are shown in the stereographic plots in Fig. 1. At first sight the molecules look similar in both crystal forms. A conspicuous difference is the conformation at the 6-s-bound which connects the cyclohexene ring to the polyene chain: α-APC shows a 6-s-*cis* whereas β-APC shows a 6-s-*trans* conformation. The molecular packing of the two crystal forms is depicted in Fig. 2a and b in which intermolecular contact distances less than 3.6 Å are marked. The plots also give the numbering of the carbon atoms which are used in the text and the following tables. Table 2 lists comparable Cartesian coordinates for the non-hydrogen atoms in the α- and β-APC-crystal form[1] whereas in Table 3 planes are defined which are fitted to various parts of the APC-molecules and the angles which exist between these planes.

α-APC: Most strikingly, the crystallographic plot in Fig. 2a reveals a polar packing of the molecules in the α-APC crystal on a two fold screw axis. The aldehyde groups are quite close to this axis with distances of 0.975(2) and 0.620(2) Å for C(23) and O, respectively. The C$=$O bonds have angles of 28.26° with the screw axis. The shortest intermolecular distance $^{\times}$O … C(21) of 3.271(2) Å corresponds to a van der Waals contact (3.25 Å). Obviously, the oxygen atom $^{\times}$O touches the π-system at C(21), as it is 2.57 Å above the plane C(20)$-$C(21)$=$C(22). The vector $^{\times}$O … C(21) forms an angle of 52° with that plane. The distance $^{\times}$O … H(21) of 2.77 Å is longer than expected for a van der Waals contact (2.6 Å). The same is true for the distances $^{\times}$O … $^{\square}$H(11) and $^{\times}$O … $^{\diamond}$H(282), both of 2.72 Å.

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[1] The complete crystallographic parameter sets are available on request from the Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK

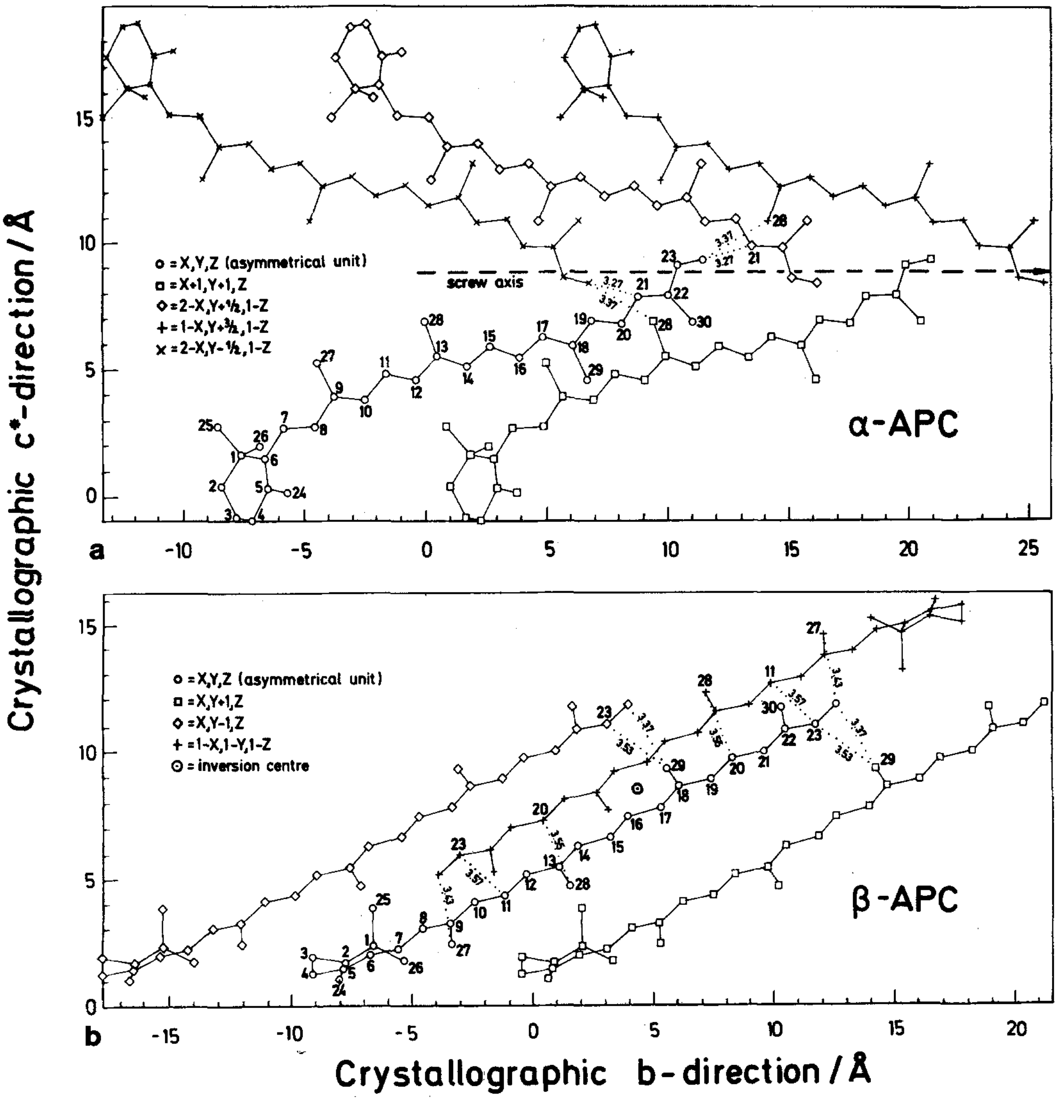


Fig. 2 Molecular packing of the C-atoms in β-8′-apocarotenal in its crystal forms α-APC and β-APC shown as projections on the c*, b-plane. The numbering of the carbon atoms is introduced at the molecule in the asymmetric unit (o). Intermolecular distances shorter than 3.6 Å are plotted

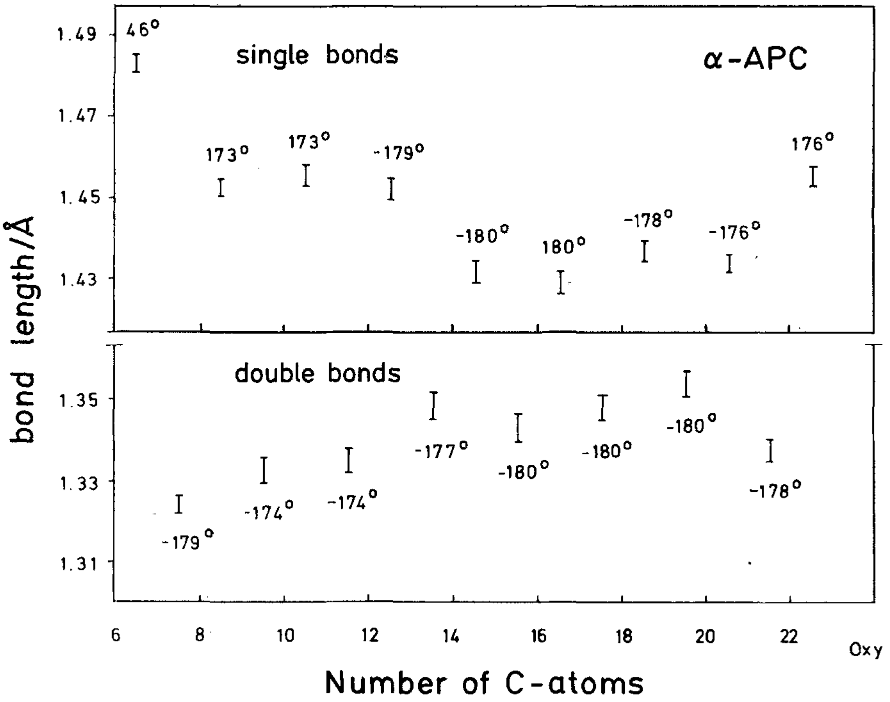
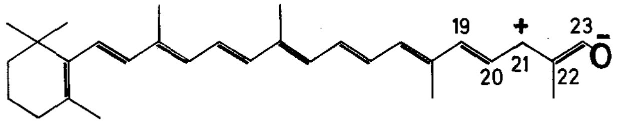


Fig. 3. C-C bond lengths within the polyene chain C(6) to 0 of α-APC, represented by *bars* of length 4σ Å plotted as a function of their position in the chain. For each bond the dihedral angle (from Table 6, rounded to full deg.'s) is inserted in order to show the influence on the conjugation

The 6-s-*cis* conformation in the atomic sequence $-C(5)=C(6)-C(7)=C(8)-$, i.e. at the bond connecting the ring with the polyene chain, causes steric hindrance between Me(24) and C(8) and thus a twist out of planarity. This is obvious from the dihedral angle $\varphi_{5-6-7-8}=46°$ (c.f. Appendix, Table 6) which represents the deviation from planarity. The short intramolecular distance C(24)...C(8) of 3.12 Å shows the repulsion left between these atoms.

With respect to the short intermolecular contact distance $^{\times}$O ... C(21) we suggest that a positive charge is stabilized at C(21) which might favor energetically the following short-binding resonance structure:



It has to be noted, however, that the C−C distances within the chain segment C(21)=C(22)−C(23) (c.f. Fig. 3) do not reflect a substantial contribution of a corresponding dipole structure.

The polyene chain C(6) to O deviates considerably from planarity. The root mean square deviation from the best plane fit (plane A in Table 3) is 0.238 Å, nearly double the value found for the corresponding plane in the *β*-APC crystal. An analysis of the deviations of the atoms defining the plane and of the dihedral angles of the bonds shows that the chain is composed of three approximately planar segments with the atoms C(6) to C(10) (plane B), C(11) to C(21) (plane C), and C(22)−C(23)=0 (plane D), respectively. The central segment is rather planar, having a r.m.s-deviation of 0.016 Å. Thus the conjugation within that segment should be least hindered. This is supported by the diagram in Fig. 3 in which the C−C bond lengths in

Table 2. Cartesian coordinates[a] of the non-hydrogen atoms in *α*-APC and *β*-APC in Å

Atom	*α*-APC			*β*-APC		
	X	*Y*	*Z*	*X*	*Y*	*Z*
C(1)	0.11678	−0.49697	−13.3411	3.34182	−1.74410	−11.2806
C(2)	0.99647	−0.95869	−14.4878	4.11280	−2.28338	−12.5081
C(3)	2.32811	−1.31158	−14.1535	4.15978	−1.30961	−13.6577
C(4)	2.49926	−2.26466	−13.0393	2.76059	−1.11008	−14.1512
C(5)	1.48540	−2.04643	−11.9288	1.74290	−0.93325	−13.0478
C(6)	0.46190	−1.18468	−12.0311	2.00494	−1.16182	−11.7534
C(7)	−0.42672	−0.87731	−10.8842	0.94465	−0.87158	−10.7666
C(8)	−0.04078	−0.60063	− 9.64832	1.04770	−0.63735	− 9.46455
C(9)	−0.90862	−0.32310	− 8.51727	−0.01552	−0.37072	− 8.50491
C(10)	−0.37453	−0.19758	− 7.30295	0.29924	−0.19798	− 7.20492
C(11)	−1.07329	−0.03793	− 6.03607	−0.56832	−0.05343	− 6.07715
C(12)	−0.46060	−0.04636	− 4.85005	−0.11108	0.02027	− 4.83239
C(13)	−1.09437	−0.01286	− 3.54370	−0.87655	0.03840	− 3.58985
C(14)	−0.32660	0.00052	− 2.43531	−0.21181	0.06826	− 2.42787
C(15)	−0.79484	−0.02105	− 1.08243	−0.75580	0.02559	− 1.10817
C(16)	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
C(17)	−0.48768	−0.02427	1.34284	−0.52217	−0.03856	1.34337
C(18)	0.20981	−0.01240	2.49585	0.17293	−0.03715	2.48634
C(19)	−0.51653	−0.05135	3.73514	−0.53560	−0.04590	3.72744
C(20)	−0.00000	−0.00000	4.98570	0.00000	0.00000	4.96199
C(21)	−0.84642	−0.05635	6.14171	−0.79156	0.06343	6.17329
C(22)	−0.49362	0.05637	7.42674	−0.33264	0.19230	7.40865
C(23)	−1.56218	−0.09587	8.40300	−1.29828	0.32128	8.49599
O	−1.43797	−0.10251	9.59326	−1.05501	0.50717	9.65249
C(24)	1.77762	−2.89541	−10.7081	0.41607	−0.43102	−13.5894
C(25)	−1.32103	−0.78083	−13.7745	4.19371	−0.67297	−10.5951
C(26)	0.25279	1.02388	−13.1968	3.12141	−2.92210	−10.3522
C(27)	−2.37576	−0.16030	− 8.76910	−1.40757	−0.35651	− 9.00494
C(28)	−2.58616	−0.02120	− 3.48760	−2.36695	−0.00635	− 3.70087
C(29)	1.71261	−0.00000	2.54196	1.65256	0.00000	2.54216
C(30)	0.87615	0.27625	7.93755	1.09072	0.25387	7.82474

[a] The definition of the molecular Cartesian axes is based on three atoms in the central part of the polyene chain, this part turned out to deviate least from planarity and presumably is most rigid. – Origin in atom C(16), nearly in the center of the polyene chain, – *Z* = vector C(16) – C(20), i.e. parallel to the central part of the chain, – *X* = within the plane C(16), C(20), C(29), perpendicular to *Z*, and positive towards C(29), – *Y* = perpendicular to this plane

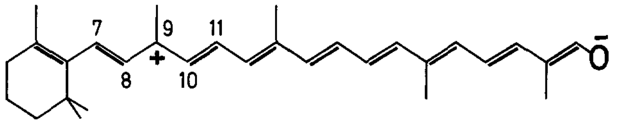
Table 3. I: Definition of planes fitted to various parts of the polyene chain of β-8′-apocarotenal (APC) in its crystal forms α-APC and β-APC, and root mean square deviations (r.m.s.d.) from the planes in Å
II: Angles between the planes in deg. (e.s.d.'s in parentheses)

I. Planes	α-APC	β-APC
(A): Chain atoms C(6) to C(23), 0	0.238	0.120 Å
(B): Chain atoms C(6) to C(10)	0.031	0.010 Å
(C): Chain atoms C(11) to C(21)	0.016	0.040 Å
(D): Chain atoms C(22), C(23), 0		
II. Angles		
Plane (A)–plane (B)	9(3)	15(3)°
Plane (A)–plane (C)	9(8)	17(4)°
Plane (A)–plane (D)	14(8)	22(3)°
Plane (B)–plane (C)	14(3)	11(1)°
Plane (B)–plane (D)	18(3)	9(1)°
Plane (C)–plane (D)	5.4(8)	9(1)°

the polyene chain are plotted as a function of their positions within the chain. Clearly, the double bonds C(13)=C(14) to C(19)=C(20) are significantly lengthened while the single bonds C(14)–C(15) to C(20)–C(21) are shortened.

It seems that most of the dipole moment in α-APC is retained in the aldehyde group. Thus strong Coulomb forces can be expected between these groups along the screw axis (see Fig. 2a). Between the polyene chains which protrude far out from this axis only attractive van der Waals forces should prevail and these might be slightly weakened by a small positive charge distributed on the chains. This may explain the relatively low density calculated for the α-APC crystals (c.f. Table 1) and the fact that between the chains no intermolecular C ··· C contact closer than 3.6 Å exists. It is surprising that in spite of the absence of short contacts the polyene chains deviate much more from planarity than in the more dense packing of the β-APC crystal (see Table 3). Furthermore, strong thermal vibrations are observed which are represented in Fig. 1 by 50% probability ellipsoids and, obviously, are always bigger than in β-APC. To our knowledge α-APC is the first carotenoid structure for which a polar packing of the molecules in the crystal was found. The crystal as a whole should have an electric moment. Furthermore, a crystal without inversion center is expected to have optical activity for all directions of incident light that are not perpendicular to the *b*-axis. Such a crystal cannot have a racemic structure and thus consists of R- or S-enantiomers only.

β-APC: Molecules with large dipole moments are usually packed in such a way that the moments of neighbouring molecules are antiparallel. In contrast to α-APC the crystal form β-APC is a typical example of antiparallel packing. In the center of Fig. 2b such a dipole pair which is related by an inversion center is shown. The close intermolecular contact distances within this 'dimer' prove clearly the electric attraction between the two antiparallel dipoles. From Fig. 2b one would expect the positive end of the dipole around te chain atom C(9) although its distance to $^{+}$0 with 3.73(1) Å is 0.5 Å longer than expected for a van der Waals contact. However, as electric forces vary much less with distance than do van der Waals forces it is not unlikely that a positive charge is indeed stabilized around C(9). It is striking that the bond C(9)=C(10) belongs to the longest double bonds and C(10)–C(11) to the shortest single bonds in the polyene chain (c.f. Appendix, Table 6). Moreover, since the specific interaction ^{x}O–C(21) of α-APC (Fig. 2a, 3.27 Å) is missing a long binding resonance structure, which reaches up to the ring, is concluded for the β-APC form:



Owing to the low accuracy of the structure determination of β-APC, however, the evidence of the two structural arguments for this conclusion is limited. – For the same reason a diagram like Fig. 3 cannot be prepared and discussed for β-APC.

The dimer packing is characteristic for carotenoids and occurs in the all-*trans* and 11-*cis* retinal crystals also (Hamanaka et al. 1972; Gilardi et al. 1972; Drikos et al. 1981). The elementary cell contains a second pair of molecules which can be derived from the first pair by a twofold screw axis in the crystallographic *b*-direction. Thus, the β-APC crystal consists of two racemic sequences of R- and S-enantiomers which are related to each other by a screw axis.

The double bond of the ring shows a 6-s-*trans* like conformation relative to the first double bond of the side chain. This is described by the dihedral angle $\varphi_{5-6-7-8}$ for which a value of −158.4(8)° is found (Table 6). The deviation from planarity i.e. −180° is thus 21.6° which is smaller than in the case of the α-form. The torsion of the ring is caused by the close contacts of C(24) to C(7) with 2.91(2) Å and to H(7) with 2.47(6) Å, respectively.

The smaller deviation from planarity for the 6-s-*trans* conformation of β-APC as compared with that for the 6-s-*cis* conformation of α-APC agrees well with the structural data found for other carotenoids (c.f. Stam 1972).

As in other carotenoids the relief of steric crowding is facilitated by slight distortions of the bond angles (see Fig. 1 and Appendix, Table 6). Whenever a methyl group is attached to the carotenoid chain the angle opposite the methyl group is reduced from the average of 126° to 119°. As a consequence the chain assumes an S-like shape which is obvious from the stereo plots of

Fig. 1 and especially from the projection of α-APC in Fig. 2a. In contrast to α-APC, in the β-APC crystal form 3 short intermolecular C ... C distances exist which are smaller than 3.6 Å (c.f. Fig. 2b and Appendix, Table 5). The oxygen atom, however, shows an intermolecular distance which is even larger than the contact distances O ... C(21) and O ... C(28) in the α-APC crystal (see Fig. 2 and Appendix, Table 5).

In spite of the higher density of the β-APC crystal (1.050 g cm^{-3}) its melting point at 132.3° is below that of α-APC at 138.5 °C. This finding may be related to a lower stability of the 6-s-*trans* conformation in the β-APC crystal as compared to the 6-s-*cis* conformation in the α-APC crystal. Quantum-mechanical calculations of retinal molecules indicate that a 6-s-*cis* conformation is indeed more stable than a 6-s-*trans* conformation (Birge et al. 1982b).

Crystal absorption spectroscopy

The polarized absorption spectra of α- and β-APC are shown in Fig. 4a and b. The corresponding spectral data are given in Table 4. In the main absorption band, the crystal spectrum of β-APC shows a much higher dichroism with respect to the *b*-axis than that of α-APC does. This is due to the different orientation of the projection of the molecule onto the plane of measurement ((001); *a*, *b*-plane) as shown in Fig. 5a and b: In the β-APC crystal the projection of the polyene chain C(6)–O is nearly parallel to the *b*-axis whereas an angle of 30° (or 180°–30°, respectively) with the *b*-axis is found for α-APC. An angle of 30° should result in a polarization ratio of 3. The ratio of the maximum absorption E‖b versus E⊥b is 2.8 for α-APC in Fig. 4a.

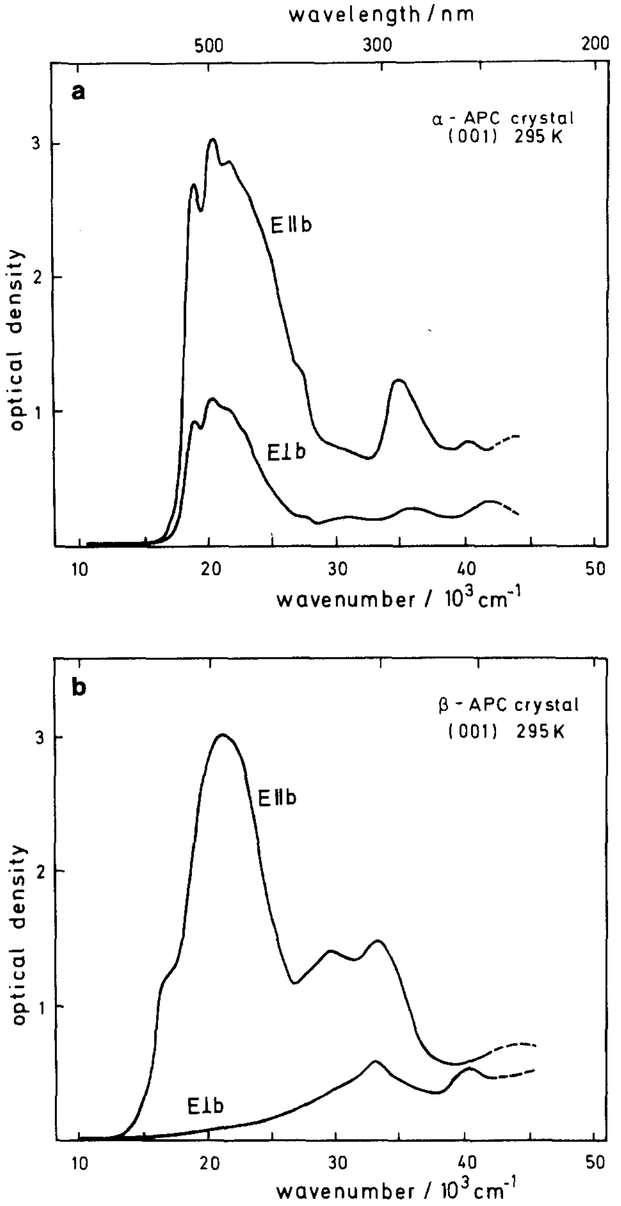


Fig. 4a and b. Polarized UV-absorption spectra of β-8′-apo-carotenal (**a**) in α-APC and (**b**) in β-APC form both measured with light incident on (001) plane. Temperature 295 K. *E*: direction of electric vector. *b*: crystallographic *b*-axis in (001) plane

The main band of the α-APC spectrum shows vibrational structure whereas that of β-APC does not (c.f. Fig. 4a and b). On the other hand, the β-APC spectrum has a clearly defined shoulder on the low energy side of the main band around 16.6 · 10^3 cm^{-1} (hereafter referred to as S-band) which is missing in the α-APC spectrum. Vibrational structure could also not be found in the main band of all-*trans* retinal and both forms of 11-*cis* retinal crystals, even not at 4.2 K. Instead, in all these cases the appearance of an S-band is observed (Drikos et al. 1984a, b). Therefore, it has been suggested that this spectroscopic property might be related to the 6-s-*cis* conformation of the retinyl ring. This conformation prevails in the retinal crystals (Hamanaka et al. 1972; Gilardi et al. 1972; Drikos et al. 1981) and also in the chromophoric site of 11-*cis* retinal in rhodopsin (Smith et al. 1987). The two crystal spectra of APC reveal spectroscopic features which seem to exclude each other: The appearance of the S-band for β-APC and a vibronic structure of the main band for α-APC. The assumed correlation with the 6-s-*cis* conformation, however, is not confirmed with APC: α-APC has a 6-s-*cis* conformation and yet shows no S-band but a vibronic structure instead.

On the other hand, there is a remarkable difference in the twist angles of the ring conformation of both forms: In the α-APC crystal form the retinyl ring is twisted +46° out of the plane of the polyene chain (see Table 3, plane *A*) whereas for β-APC the twist is only –21.6° out of the 6-s-*trans* conformation (see Appendix, Table 6). However, in the crystal form α-*CIS* of 11-*cis*-retinal one has a twist of +40° and in the crystal

Table 4. Spectral data of APC at 300 K

	α-APC crystal form			β-APC crystal form		APC in solution	
	Direction of polarization	Band position/10^3 cm^{-1}	Estimated polarization direction[c]	Band position/10^3 cm^{-1}	Estimated polarization direction[c]	Band position/10^3 cm^{-1}	
						EtOH	PE
Low energy shoulder (probably $^1A_g^{-}{*}$)	E ∥ b	–	–	16.6	–		
	E ⊥ b	–	–	–			
	random					–	–
Main band (probably $^1B_u^{+}{*}$)	E ∥ b	18.6 20.1 21.3 22.9[a]	31°	20.9	10°		
	E ⊥ b	18.7 20.1 21.3 22.8[a]		21,5[a]			
	random					21.6	20.9, 22.0, 23.1
First high energy band	E ∥ b	27.0	22°	27.7[a]	–		
	E ⊥ b	27.1		–			
	random					–	–
Second high energy band (probably $^1A_g^{+}{*}$)	E ∥ b	30.5	28°	29.7	27°		
	E ⊥ b	30.4		30.0[a]			
	random					–	30.1[b]
Third high energy band (probably $2\,^1B_u^{+}{*}$)	E ∥ b	34.5	25°	33.2	32°		
	E ⊥ b	35.7		33.0 36.0[a]			
	random					36.1	36.1[a] 38.1
Fourth high energy band	E ∥ b	40.0	32°	43.5[a]	40°		
	E ⊥ b	41.4		40.2			
	random					–	

[a] Not clearly defined shoulder

[b] Increasing as the sample is exposed to light

[c] Acute angle to b-axis estimated according $\pm \operatorname{arctg}\sqrt{A_{max,\parallel b}/A_{max,\perp b}}$ (see Drikos and Rüppel 1984)

EtOH: ethyl alcohol

PE: Petroleum ether, light petroleum 40 – 60 °C

of all-*trans* retinal an even larger twist of +59° out of the 6-s-*cis* position of the ring linkage. In spite of these large twist angles the corresponding crystal absorption spectra of both forms clearly show the S-band and no vibrational structure (Drikos et al. 1984a; Drikos and Rüppel 1984). Therefore, it is very unlikely that the conformation of the 6-s-linkage of the retinyl-ring has influence on the properties of the low lying electronic excitations of retinal and its derivatives.

The reason for the lack in vibronic structure and/or the appearance of the S-band might rather be found in different interactions caused by the crystal packing. It is significant that the α-APC is not so densely packed as β-APC with the exception of the aldehyde ends of the polyene chain. Only here, near the screw axis (Fig. 2a) do close contact distances exist <3.6 Å (Appendix, Table 5). In the case of β-APC, however, there is a dense inversion linked R-S-dimer packing with long parallel segments C(9)–O of the polyene chain and several close intermolecular distances over the whole segment (Fig. 2b and Appendix, Table 5). A similar packing is observed for α- and β-*CIS*-retinal (Gilardi et al. 1972; Drikos et al. 1981) and all-*trans* retinal (Hamanaka et al. 1972). Weak intermolecular vibrations between the whole molecules of this R-S-dimer in the ground level could lead to a "horizontal" broadening of the main band (Birge et al. 1982b). As a matter of fact, the crystal spectrum of all-*trans* retinal at 4.2 K scarcely shows any vibrational structure (Drikos et al. 1984b). This finding would suggest that oscillations in the case of R-S-inversion "dimers" should be very weak and result in a diffuse main absorption band. On the other hand, in the case of α-APC the limitation of the interaction to just the small molecule segment C(21)...O (Fig. 2a) should lead to the absence of weak intermolecular oscillations and thus result in a resolved main band.

Presupposing the existence of this molecular interaction the occurrence of such "dimers" could also explain the observation that in ethanol solution at room temperature the main band of APC is without vibrational structure (c.f. Takemura et al. 1978). In solutions of petroleum ether, however, such "dimers" should not occur because in this solvent the spectrum of APC shows a main band with a distinct vibrational structure.

The most remarkable finding is the shoulder on the low energy side of the main absorption band (S-band) which occurs in the crystal spectrum of β- but not of α-APC. A similar shoulder was observed in the crystal spectrum of all-*trans*-retinal on this side of the main band around $23.4 \cdot 10^3$ cm^{-1} (Drikos et al. 1984a) and at Helium temperature also in the crystal spectrum of 11-*cis* retinal at $22.6–22.9 \cdot 10^3$ cm^{-1} (Drikos et al.

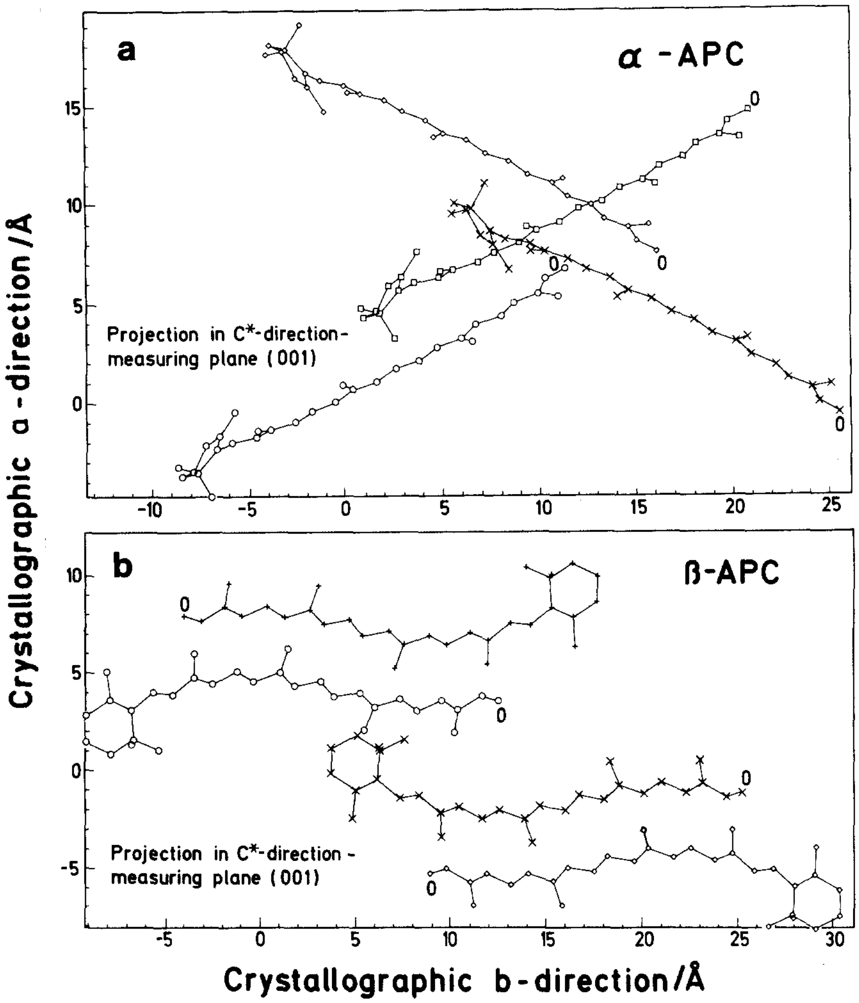


Fig. 5 a and b. Projection of the unit cell along c^*-axis onto a, b plane (001) for crystal form α-APC (**a**) and crystal form β-APC (**b**).

1984b). This band has been assigned with high probability to a transition into a covalent $^1A_g^{-*}$-state (Drikos and Rüppel 1984). A transition at $23.4 \cdot 10^3$ cm^{-1} could also be observed by measuring a two photon absorption spectrum of all-*trans*-retinal in solution (Birge et al. 1982a). As a matter of fact, the transition into the $^1A_g^{-*}$-state is forbidden for ideal polyenes in one photon absorption but is allowed for simultaneous absorption of two photons (Birge et al. 1982a). However, if the ideal π-electron structure is disturbed, for instance by introducing a carbonyl group then ionic B_u^{+*}-character is added to the $^1A_g^{-*}$-state so that a perceptible one photon absorption becomes possible (Birge and Pierce 1979). By analogy, the shoulder on the low energy side of the main band in the β-APC crystal spectrum could also be interpreted by a transition into the $^1A_g^{-*}$-state. This assumption is further supported by the result of semiempirical CI-calculations on disturbed polyenes with variable numbers of double bonds (Schulten et al. 1980). According to these calculations the close neighbourhood (3.4–4.0 Å) of two parallel π-electron systems which are linked to each other by an inversion center should cause a considerable admixture of B_u^+-character to the $^1A_g^{-*}$-state. As such a constellation of two π-electron systems is absent in the α-APC crystal the $^1A_g^{-*}$-state can presumably not be observed in its spectrum at room temperature. A comprehensive answer to these questions could be expected by repeating the spectral measurements at Helium temperatures as well as by double CI-calculations of the "inversion dimer" of the β-APC crystal.

An alternative interpretation of this shoulder could be a charge transfer transition (Mulliken and Person 1969). Such an electron transfer could take place from the carbonyl group of one molecule of the inversion linked dimer as donor to the conjugated part of the ring in the other molecule as acceptor. This interpretation of the S-band, however, seems to be not very likely: Firstly, such a charge transfer transition should be perpendicular to the axis of the dimer which contradicts the experimental results. Secondly, such a charge transfer transition between the same type of molecules should be too weak to be directly observed in absorption.

The energy separation between the S-band and the main band (see Table 4) amounts to $3.3 \cdot 10^3\ \mathrm{cm}^{-1}$ for the β-APC in comparison to $4.1 \cdot 10^3\ \mathrm{cm}^{-1}$ in the case of all-*trans* retinal (Drikos et al. 1984a). If the S-band is assigned to a transition into the ${}^1A_g^{-*}$-state, according to a recent PPP-MRD-CI-study of Tavan and Schulten (1986) the energy separation between ${}^1B_u^+$ and ${}^1A_g^{-*}$ should decrease for polyenes with increasing chain length. Between all-*trans* retinal (8 C-atoms in the chain) and apocarotenal (17 C-atoms) the decrease should be 32%, in fairly good accordance with 20% found here in the crystal spectra.

As for the high energy bands of the β-APC crystal spectrum (see Fig. 4 and Table 4) the band at approximately $30 \cdot 10^3\ \mathrm{cm}^{-1}$ can readily be recognized as a *cis*-peak and thus an assignment be proposed to the ${}^1A_g^{+*}$-state. This assignment is suggested by comparing the crystal spectrum with an illuminated solution spectrum of APC. As a matter of fact, it is the band at $30.1 \cdot 10^3\ \mathrm{cm}^{-1}$ which is increased after illumination (c.f. Table 4). In that case, the following high energy band should be a second $\pi\pi^*$-transition into a B_u^{+*}-state.

The high energy shoulder of the main band in the α-APC-spectrum at approximately $27 \cdot 10^3\ \mathrm{cm}^{-1}$ could be a further *cis*-band which is related to the 6-s-*cis* bond of this conformer. With non-parallel packing of the molecules produced by a screwing operation a charge transfer transition would be hardly imaginable.

Summarizing, from the crystal spectra of β-8′-Apocarotenal at room temperature at least six energy transitions are recognized. Relative to the position of the main transition at $20.9 \cdot 10^3\ \mathrm{cm}^{-1}$ one low energy and four high energy transitions are observed. The proposed but still tentative band assignments are listed in Table 4. For an improved interpretation of these bands it is necessary to have more experimental data especially from low temperature crystal spectra of β-8′-carotenal and its *cis*-isomers which have not been available yet for these investigations. – The observation of a low lying electronic state – probably ${}^1A_g^-$ - for β-APC only might be of significance for the understanding of the photodynamic behavior of the visual chromophore. The crystal form β-APC but not α-APC shows a strong electrostatic interaction between pairs of molecules in the unit cell (c.f. Figs. 2 and 5). For the retinylidene chromophore, in order to interpret the large static red shift of the main absorption band, corresponding electrostatic interactions are postulated to exist between the π-electron system of the polyene chain and the protein helices forming its binding site.

Acknowledgements. This work was supported by the Deutsche Forschungsgemeinschaft within the frame-work of the special project "Visual chromophore" including a personal grant to one of the authors (G.D.). A financial support of the Fond der Chem. Industrie, Frankfurt, is also greatly acknowledged. The authors wish to thank Dr. G. Chatzitheodorou for his assistance in crystallizing the β-APC form of β-8′-Apocarotenal from nonpolar solvents.

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Appendix

Table 5. Intermolecular distances in Å (< 3.6 Å) of α-APC and β-APC

α-APC		β-APC	
O ··· C(21) (screwing)	3.27	O ··· C(29) (translation)	3.37
O ··· C(28) (screwing)	3.37	O ··· C(27) (inversion)	3.43
		C(20) ··· C(28) (inversion)	3.55
		C(23) ··· C(11) (inversion)	3.57
		C(23) ··· C(29) (translation)	3.53

Table 6. Bond length (*I*) in Å, bond angles (*II*), and dihedral angles (*III*) in degrees of β-8′-apocarotenal (APC) in its crystal forms α- and β-APC at 290 K (e.s.d.'s in parentheses). Comparison with equivalent parameters of 11-*cis*-12-s-*cis*-retinal in its crystal forms α-*CIS* (Gilardi et al. 1972) and β-CIS (Drikos et al. 1981)

	Atoms	α-APC	β-APC	α-*CIS*	β-*CIS*
I.	C(1) – C(2)	1.517(1)	1.55(2)	1.523	1.52(1) Å
	C(1) – C(6)	1.519(1)	1.53(2)	1.528	1.563(8) Å
	C(1) – C(25)[a]	1.528(2)	1.53(2)	1.528	1.55(1) Å
	C(1) – C(26)[a]	1.534(2)	1.52(1)	1.563	1.52(1) Å
	C(2) – C(3)	1.418(2)	1.51(2)	1.498	1.50(1) Å
	C(3) – C(4)	1.476(2)	1.50(2)	1.532	1.53(1) Å
	C(4) – C(5)	1.520(1)	1.51(1)	1.521	1.50(1) Å
	C(5) = C(6)	1.342(1)	1.34(1)	1.333	1.328(9) Å
	C(5) – C(24)[a]	1.515(2)	1.52(2)	1.500	1.526(8) Å
	C(6) – C(7)	1.483(1)	1.48(1)	1.486	1.461(9) Å
	C(7) = C(8)	1.324(1)	1.33(1)	1.339	1.350(9) Å
	C(8) – C(9)	1.452(1)	1.46(1)	1.461	1.43(1) Å
	C(9) = C(10)	1.333(2)	1.35(1)	1.347	1.356(9) Å
	C(9) – C(27)[a]	1.497(1)	1.48(1)	1.511	1.503(9) Å
	C(10) – C(11)	1.456(1)	1.43(1)	1.454	1.43(1) Å
	C(11) = C(12)	1.335(2)	1.33(1)	1.339	1.347(9) Å
	C(12) – C(13)	1.452(1)	1.46(1)	1.472	1.48(1) Å
	C(13) = C(14)	1.348(2)	1.34(1)	1.358	1.32(1) Å
	C(13) – C(28)[a]	1.493(1)	1.50(1)	1.526	1.525(9) Å
	C(14) – C(15)	1.432(1)	1.43(1)	1.467	1.47(1) Å
	C(15) = C(16)	1.343(2)	1.34(1)		
	C(16) – C(17)	1.429(1)	1.44(1)		
	C(17) = C(18)	1.348(2)	1.34(1)		
	C(18) – C(19)	1.437(1)	1.43(1)		
	C(18) – C(29)	1.504(1)	1.48(1)		
	C(19) = C(20)	1.354(2)	1.35(1)		
	C(20) – C(21)	1.434(1)	1.45(1)		
	C(21) = C(22)	1.337(1)	1.32(1)		
	C(22) – C(23)	1.455(1)	1.46(1)		
	C(22) – C(30)	1.478(1)	1.48(2)		
	C(23) = O[a]	1.197(2)	1.20(1)	1.213	1.17(1)
II.	C(2) – C(1) – C(6)	112.48(7)	108.8(9)	111.5	110.5(6)°
	C(2) – C(1) – C(25)[a]	105.9(1)	109(1)	111.4	111.8(6)°
	C(2) – C(1) – C(26)[a]	108.75(8)	106.7(8)	106.9	107.9(6)°
	C(6) – C(1) – C(25)[a]	111.97(9)	111.0(8)	109.7	107.8(6)°
	C(6) – C(1) – C(26)[a]	110.34(9)	110.9(9)	111.2	110.7(5)°
	C(25) – C(1) – C(26)[a]	107.11(9)	111(1)	106.1	108.1(6)°
	C(1) – C(2) – C(3)	116.2(1)	113.3(9)	112.1	113.4(7)°

Table 6 (continued)

Atoms	α-APC	β-APC	α-*CIS*	β-*CIS*
C(2) – C(3) – C(4)	116.60(9)	108(1)	111.9	109.9(7)°
C(3) – C(4) – C(5)	112.43(8)	114(1)	113.3	114.0(6)°
C(4) – C(5) = C(6)	123.05(8)	124(1)	122.9	124.9(6)°
C(4) – C(5) – C(24)[a]	112.31(8)	111.5(9)	111.3	112.2(6)°
C(6) = C(5) – C(24)[a]	124.63(8)	124.9(9)	125.8	122.9(6)°
C(1) – C(6) = C(5)	121.98(8)	122.2(9)	122.8	120.8(6)°
C(1) – C(6) – C(7)	115.91(6)	119.6(8)	114.0	114.1(5)°
C(5) = C(6) – C(7)	122.08(8)	118.1(9)	123.2	125.2(6)°
C(6) – C(7) = C(8)	126.19(7)	129.4(9)	126.2	128.5(6)°
C(7) = C(8) – C(9)	126.35(8)	129(1)	126.4	127.3(6)°
C(8) – C(9) = C(10)	119.21(7)	119.2(9)	117.8	120.1(6)°
C(8) – C(9) – C(27)[a]	118.38(9)	117.8(8)	117.9	118.7(5)°
C(10) = C(9) – C(27)[a]	122.40(8)	123.0(8)	124.3	121.2(6)°
C(9) = C(10) – C(11)	127.66(8)	129.2(9)	125.3	126.3(6)°
C(10) – C(11) = C(12)	123.52(8)	122.4(9)	128.1	129.5(7)°
C(11) = C(12) – C(13)	126.78(8)	128.2(9)	129.9	127.5(7)°
C(12) – C(13) = C(14)	119.42(8)	118.6(9)	121.3	123.6(6)°
C(12) – C(13) – C(28)[a]	118.02(9)	117.3(8)	114.0	111.9(6)°
C(14) = C(13) – C(28)[a]	122.55(8)	124.1(8)	124.7	124.4(7)°
C(13) = C(14) – C(15)	126.19(8)	127.7(9)	122.9	127.4(6)°
C(14) – C(15) = C(16)	124.60(8)	123.3(9)		
C(15) = C(16) – C(17)	123.72(8)	125(1)		
C(16) – C(17) = C(18)	128.85(8)	127(1)		
C(17) = C(18) – C(19)	118.43(7)	119.0(9)		
C(17) = C(18) – C(29)	122.93(8)	123.5(8)		
C(19) – C(18) – C(29)	118.62(9)	117.6(8)		
C(18) – C(19) = C(20)	127.06(8)	127(1)		
C(19) = C(20) – C(21)	121.19(8)	123.4(9)		
C(20) – C(21) = C(22)	128.00(8)	127(1)		
C(21) = C(22) – C(23)	116.24(8)	118(1)		
C(21) = C(22) – C(30)	126.08(7)	126.7(9)		
C(23) – C(22) – C(30)	117.65(9)	115.0(8)		
C(22) – C(23) = O[a]	126.3(1)	127(1)	121.4	126.1(7)°
III. C(5) = C(6) – C(1) – C(2)	3.3(1)	−13(1)	−18.8(7)	−17.2(9)°
C(5) = C(6) – C(1) – C(25)	122.4(1)	107(1)		
C(5) = C(6) – C(1) – C(26)	−118.4(1)	−129.7(9)		
C(7) – C(6) – C(1) – C(2)	−174.93(8)	165.6(7)		
C(7) – C(6) – C(1) – C(25)[a]	−55.8(1)	−74(1)	−74.3(5)	−74.8(7)°
C(7) – C(6) – C(1) – C(26)[a]	63.45(9)	49(1)	42.8(6)	43.2(8)°
C(6) – C(1) – C(2) – C(3)	−32.6(1)	48(1)	47.4(7)	48.3(9)°
C(25) – C(1) – C(2) – C(3)	−155.2(1)	−73(1)		
C(26) – C(1) – C(2) – C(3)	89.9(1)	168(1)		
C(1) – C(2) – C(3) – C(4)	51.0(1)	−65(1)	−59.1(7)	−60(1)°
C(2) – C(3) – C(4) – C(5)	−36.9(1)	44(1)	39.8(7)	40(1)°
C(3) – C(4) – C(5) = C(6)	7.1(1)	−11(1)	−11.5(8)	−11(1)°
C(3) – C(4) – C(5) – C(24)	−172.2(1)	167.3(8)		
C(4) – C(5) = C(6) – C(1)	8.8(1)	−5(1)	1.1(7)	−1(1)°
C(4) – C(5) = C(6) – C(7)	−173.13(8)	176.4(7)		
C(24) – C(5) = C(6) – C(1)	−171.9(1)	176.5(8)		
C(24) – C(5) = C(6) – C(7)	6.1(2)	−2(1)	3.9(8)	2(1)°
C(5) = C(6) – C(7) = C(8)	46.2(1)	−158.4(8)	41.4(7)	40(1)°
C(1) – C(6) – C(7) = C(8)	−135.59(9)	23(1)		
C(6) – C(7) = C(8) – C(9)	−178.80(7)	−179.1(8)	179.5(4)	−176.1(7)°
C(7) = C(8) – C(9) = C(10)	173.11(8)	177.9(8)	170.8(5)	170.0(7)°
C(7) = C(8) – C(9) – C(27)[a]	−8.1(1)	0.3(9)	−7.2(7)	−9(1)°
C(8) – C(9) = C(10) – C(11)	−174.18(8)	−173.5(7)	−175.5(4)	−173.8(7)°
C(27) – C(9) = C(10) – C(11)	7.1(1)	4(1)		
C(9) = C(10) – C(11) = C(12)	173.39(8)	175.3(8)	−179.3(5)	−176.6(8)°
C(10) – C(11) = C(12) – C(13)	−174.12(8)	−173.6(7)	2.1(9)	8(1)°
C(11) = C(12) – C(13) = C(14)	−178.65(9)	177.6(8)	38.7(9)	39(1)°
C(11) = C(12) – C(13) – C(28)	2.5(1)	−1(1)		
C(12) – C(13) = C(14) – C(15)	−177.32(8)	−176.7(7)	179.8(5)	176.8(8)°
C(28) – C(13) = C(14) – C(15)	1.5(1)	2(1)	3.1(9)	0(1)°

Table 6 (continued)

Atoms	α-APC	β-APC	α-*cis*	β-*cis*
C(13) = C(14) − C(15) = C(16)	−179.65(9)	176.9(8)		
C(14) − C(15) = C(16) − C(17)	−179.90(8)	179.8(7)		
C(15) = C(16) − C(17) = C(18)	179.70(9)	−178.5(8)		
C(16) − C(17) = C(18) − C(19)	−179.62(8)	178.5(7)		
C(16) − C(17) = C(18) − C(29)	−1.4(1)	−0(1)		
C(17) = C(18) − C(19) = C(20)	−177.51(8)	−177.6(8)		
C(29) − C(18) − C(19) = C(20)	4.2(1)	1(1)		
C(18) − C(19) = C(20) − C(21)	−179.64(8)	176.4(7)		
C(19) = C(20) − C(21) = C(22)	−175.99(8)	−175.6(8)		
C(20) − C(21) = C(22) − C(23)	−177.54(8)	176.2(8)		
C(20) − C(21) = C(22) − C(30)	0.4(1)	−2(1)		
C(21) = C(22) − C(23) = O[a]	175.61(9)	−176.2(8)	−174.5(7)	−175(1)°
C(30) − C(22) − C(23) = O	−2.5(1)	2(1)		

[a] In the case of α- or β-*CIS* substitute atom C(n) for atom C(m). m/n: 22/14; 23/15; 24/16; 25/17; 26/18; 27/19; 28/20