A SECOND CRYSTAL FORM OF 11-*CIS*,12-*CIS* RETINAL, THE CHROMOPHORIC GROUP IN VISUAL PIGMENTS

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1. Introduction

The primary step in the visual process is caused by light absorption in 11-*cis* retinal which forms the chromophore of all known visual pigments [1]. The photoisomerization of the excited molecule into the all-*trans* configuration is thought to initiate the dark reaction sequence which constitutes the phototransduction process [2]. It seems that the considerable sterical hinderance in the polyene chain of the chromophore and the large wavelength dependent quantum yield for *cis* → *trans* photoisomerization are most important for the biophysical function of the visual chromophore. An understanding of the photophysics of the isolated chromophore should therefore be helpful for the interpretation of the initial steps of visual excitation. Consequently, a considerable number of experimental [3–9] and theoretical [10–12] investigations have been carried out on this subject. However, the relative order and nature of the low-lying electronic states involved in the photophysics of the molecule as well as the proper assignment of its high wavenumber transitions are still subject to controversy [7,12]. Absorption spectra in the gaseous or liquid state, i.e., from disoriented molecules possibly involving different conformations [3,13–15], are rather unlikely to explain the photophysics of this particular isomer.

Recently, we took another approach to the solution of this problem when the crystallization of very thin (ca. 0.2 μm) monocrystal platelets of retinal isomers became possible. With these samples we were able to obtain, for the first time, polarized absorption spectra of exactly oriented retinal molecules. During these investigations three types of crystal platelets, including a new crystal form of this isomer, were, unexpectedly, found. Hence, a combined spectroscopical and crystallographical study of more than one crystal form of 11-*cis* retinal may answer the questions about this compound, especially with regard to its biological function in the visual pigment, rhodopsin.

In this communication a new crystal form is presented and compared with the polymorph reported by Gilardi et al. [16]. The fact that only the 12-*s*-*cis* conformer can be crystallized is discussed with respect to its most probable conformation in rhodopsin. The results of the micro-spectrophotometrical studies and the spectroscopic conclusions will be given in a following paper.

2. Materials and methods

The 11-*cis* retinal was synthesized from commercially available *trans*-retinal as described before [16]. Sufficiently large crystal platelets (ca. 0.4 × 0.2 × 0.1 mm) were grown in beaker covers from polycrystalline material in butanol at −18°C. Using a polarizing microscope, the different crystal platelets were identified by their shape and their conoscopical properties.

Crystallographical X-ray measurements were carried out for each of the different crystal platelets. The X-ray data were taken from an automatic Nonius diffractometer. For this purpose, the latter had to be installed in a light-tight box. The retinal crystals were fixed onto a glass foil of 0.02 mm thickness by means of a tiny drop of petroleum ether. During the measurements the crystals were kept in a flow of dry

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nitrogen at 17–18°C to protect the crystals from air.

For the structure determination, 48 204 reflection profiles were gathered up to $\sin\theta/\lambda = 0.7031$ Å^{-1} over a period of 400 h. During this time the intensity of the reference reflection decreased to 15% of its initial value. The thin glass foil supporting the crystal showed a changing deformation in the nitrogen flow, thus influencing the accuracy of the measurements. Therefore, the overall quality of the reflection data is relatively poor but still comparable to that of Gilardi et al. [16].

The phase problem was solved with the program MULTAN [17]. The refinement of the atomic parameters was based on the structure factor (F^2) and carried out with the program X-RAY [18]. The final discrepancy index $R(F^2)$ was 0.127.

3. Results

3.1. *The crystals of 11-*cis *retinal*

More than 80% of the crystals of 11-*cis* retinal obtained in each crystallization experiment have the form described by Gilardi et al. [16], which crystallizes on the (001) face. This form will be referred to hereafter as *α-cis*. The remaining crystal types represent another polymorph of 11-*cis*,12-*s-cis* retinal which crystallizes on both $(10\bar{1})$ and (001) faces, and will be called *β-cis* in the following.

The space group of *β-cis* is $P2_1/n$ with 4 molecules per elementary cell. The cell parameters are a = 13.180(5)Å, b = 7.499(2)Å, c = 17.803(9)Å, β = 95.66(4)° with a cell volume V = 1749 Å^3.

3.2. *Molecular and crystal structure*

The geometry of the molecule is nearly the same in both crystal forms. The angles between the three planar segments of the conjugated electron system [16] represented by the dihedral angles along the C6–C7 and C12–C13 bond, respectively, (see fig.1) are, within 2°, the same in both crystal forms. If the differences occurring in bond lengths $1_\alpha - 1_\beta$ (in Å), bond angles $\phi_\alpha - \phi_\beta$ and torsion angles $\theta_\alpha - \theta_\beta$ (circular arrows) are larger than 2σ they are marked in the molecule scheme of fig.1. The essential difference between both polymorphs is discernible only by the packing of the molecules.

For both crystal forms the dipole packing of the

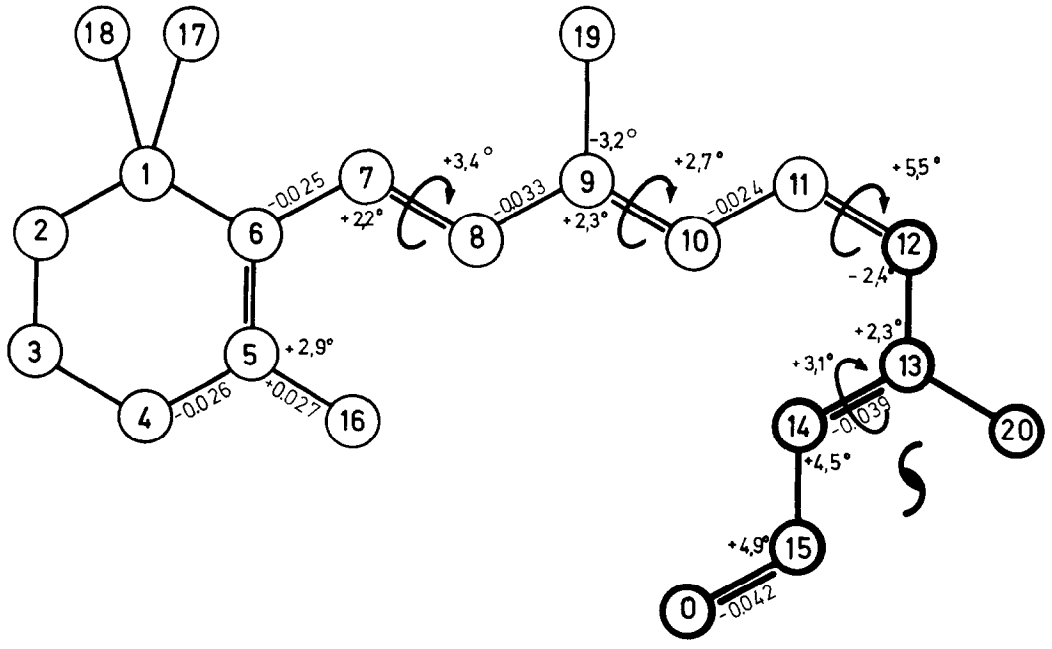

Fig.1. Schematic drawing of 11-*cis*,12-*s-cis* retinal with the usual numbering of C atoms and a notation of significant differences in bond lengths $1_\alpha - 1_\beta$ (in Å), bond angles $\phi_\alpha - \phi_\beta$, and torsion angles $\theta_\alpha - \theta_\beta$ found between the *α-cis* and *β-cis* form. Note the uniform shortening of the single bonds in segment C6–C12 and the shortening of the double bonds in the aldehyde segment. Larger differences in torsion angles θ occur, particularly along double bonds. The position of the screw axis is marked by the 'thickened S-type' symbol, see text.

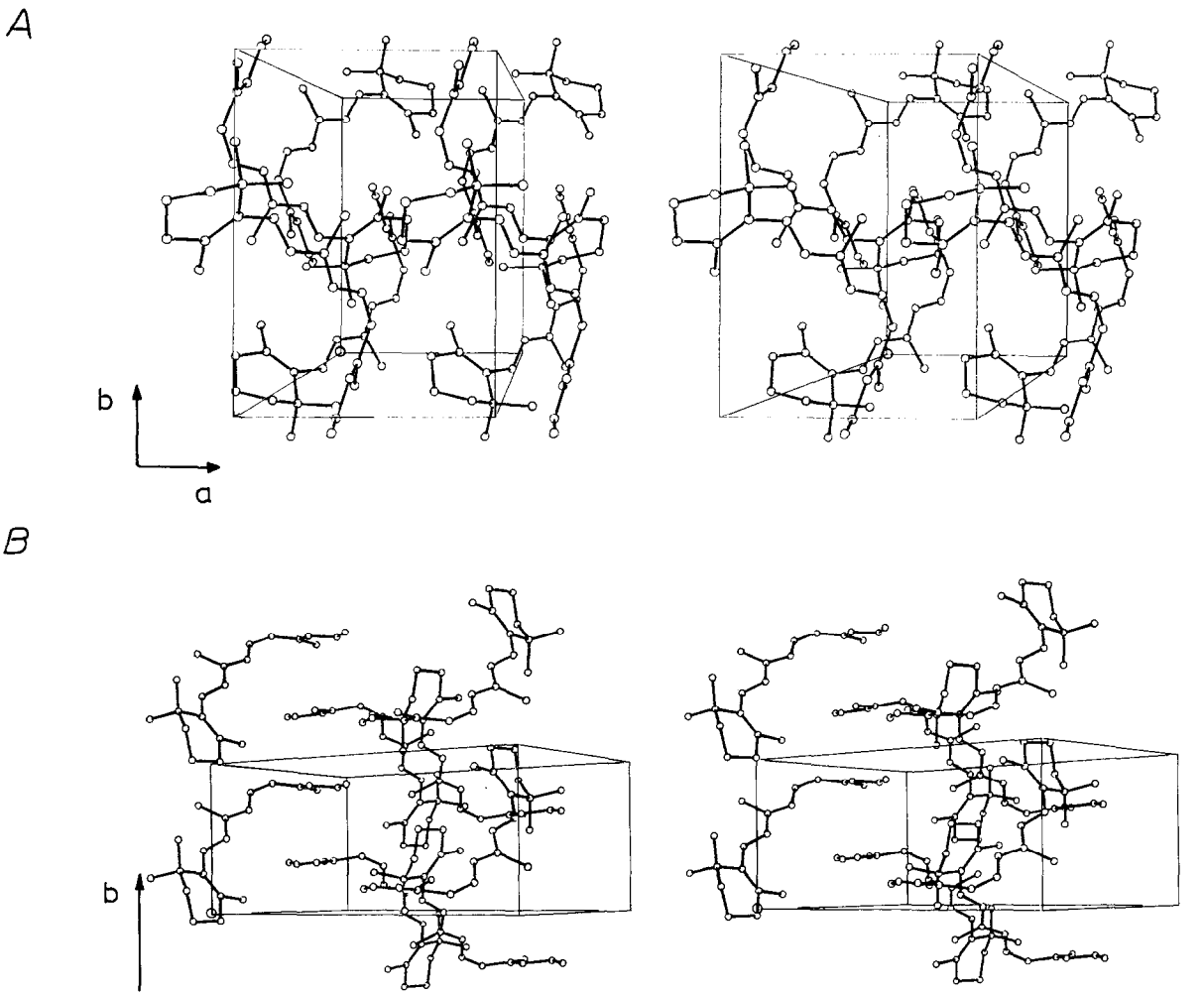

Fig.2(A). Stereopacking diagram of α-*cis* retinal on the (001) face. The origin is marked by a circle. Arrows indicate the direction of *a* and *b* axis. (B) Stereopacking diagram of β-*cis* retinal on the (10$\bar{1}$) face. The origin is marked by a circle. Arrow indicates the direction of the *b* axis.

aldehyde segment is important. These segments are packed in infinite segments with antiparallel orientation of neighbouring dipoles. Since the aldehyde segments are planar (cf. the heavier line in fig.1) a periodic sequence of this kind can best be generated either by a screw axis perpendicular to the segment plane ($\mathcal{S}$ in fig.1; parallel to the *b*-axis in fig.2B) or by inversion centers between neighbouring segments. The first possibility leads to the β-*cis*, the second to the α-*cis* form.

The similarity in the packing is thus confined to the planar aldehyde segments only, which is the origin of the dipole forces. However, for the rest of the molecule the replacement of the screw axis by inversion centers results in a different arrangement of both enantiomers of 11-*cis* retinal which are present in these crystals. This means, in the α-*cis* polymorph, the dipole sequences which form the crystal are racemic 'R, S-sequences'. In the β-*cis* polymorph, however, each dipole sequence consists of one enantiomer only, i.e., the enantiomers are separated into 'R-sequences' and 'S-sequences' which are related by inversion centers between the whole sequences.

3.3. *Comparison of structure projections of* α- *and* β-cis *crystals*

A remarkable relation exists between the structure projections of α- and β-*cis* polymorphs on faces (001)

and $(10\bar{1})$, respectively. Platelets with planes along these faces are found in the crystallization experiments (fig.2A and B). By selecting one appropriate molecule from the *α-cis* (001) and one from the *β-cis* $(10\bar{1})$ stereodiagram, one can easily ascertain that the corresponding projections of the molecules are nearly identical. In addition, bringing the *a*-axis of the *α-cis* in coincidence with the *b*-axis of the *β*-cis stereodiagram, enables one to recognize that the molecule projection in the *α-cis* packing is rotated clockwise by approximately 10° against the projection of the molecule in the *β-cis* packing. Just this small rotation has been proven to be important for the unequivocal band assignment of polarized crystal spectra of 11-*cis* retinal. This subject will be discussed in another paper.

4. Discussion

In order to explain the temperature dependence of the 11-*cis* retinal absorption spectrum a further conformer, 11-*cis*,12-*s-trans* retinal is generally postulated in the literature which should be in thermal equilibrium with 11-*cis*,12-*s-cis* retinal [15].

There are, however, some aspects which are difficult to reconcile with this assumption. First, to explain the equal low-temperature position of the absorption maxima being nearly equal for all retinal isomers, the postulated 12-*s-trans* molecules was presumed to be planar [13], or, roughly planar [14] rotated 30° about the C12–C13 bond to compensate for its blue shift created by the missing second *cis* bond [13]. (According to *π*-electron theory, the replacement of a *cis* by a *trans* bond as well as a deviation from planarity causes a blue shift of the absorption maximum.) The absorption maximum of the 11-*cis*,14-methyl retinal (which was especially synthesized to enforce a 12-*s-trans* conformation) is still blue shifted by 25 nm relative to the other 14-methyl isomers [6]. Second, a direct experimental proof for the existence of a *stable* 12-*s-trans* conformation cannot be given yet [5,19,20].

In agreement with these considerations, additional arguments from our investigations favour the distorted 12-*s-cis* conformations to be the ground state geometry of 11-*cis* retinal and the 12-*s-trans* conformational forms to be not quite so stable. First, at room temperature the 12-*s-trans* conformer is estimated to be 65% predominant in the postulated equilibrium [3]. Therefore, it would be reasonable to expect that at least a small percentage of this conformer should also crystallize. However, when 11-*cis* retinal was crystallized in different polar and non-polar solvents no other crystal types than those described in this communication could be detected. Second, according to the large sterical flexibility of 11-*cis*,12-*s-cis* retinal, large distortions around the single bonds should be expected between its two crystal forms [21,22]. Since such distortions are not observed, the difference between the intermolecular forces in both forms have almost no effect on the geometry of the molecule. Consequently the 11-*cis*,12-*s-cis* conformation described above should possess a remarkable stability.

Although these stability considerations cannot be fully convincing they may act as a warning against the general assumption, prevailing in the literature, of a 12-*s-trans* conformation for the retinal in rhodopsin. Moreover, since a conclusive assignment of the retinal conformation in rhodopsin has not yet been given [23,24] the possible presence of a distorted 12-*s-cis* geometry should be alternatively considered for the structure and photophysics of rhodopsin.

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