

The Crystal and Molecular Structure of 11-*cis*-Retinal

BY R. D. GILARDI, I. L. KARLE AND J. KARLE

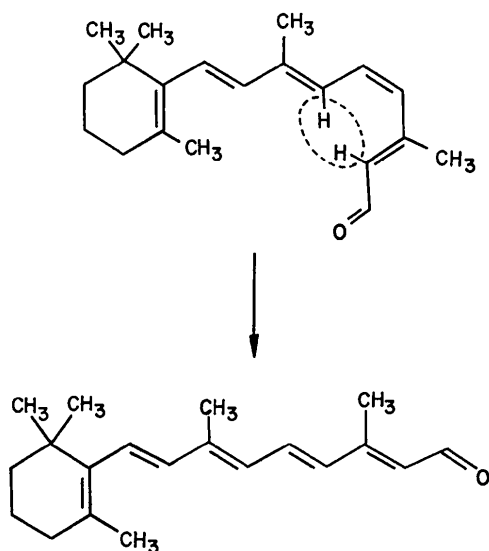
Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C., 20390, U.S.A.

(Received 13 March 1972)

The primary biochemical event in the visual response process is the isomerization of 11-*cis*-retinal ($C_{20}H_{28}O$) to all-*trans*-retinal. The space group of the 11-*cis*-retinal crystal is $P2_1/c$, with four molecules in a unit cell of dimensions $a = 7.540 \pm 0.004$, $b = 10.666 \pm 0.006$, $c = 22.102 \pm 0.020$ Å, $\beta = 95.19 \pm 0.06^\circ$, and density $D_x = 1.07$ g.cm⁻³. Intensity data were measured on an automatic diffractometer with Cu $K\alpha$ (1.54178 Å) radiation. Freshly prepared crystals were protected from oxygen, water vapor, and visible light during the experiment but deterioration nevertheless ensued. 2345 independent reflections were collected. Additional difficulties in the data collection arose from twinning. Phases were obtained routinely with the symbolic addition procedure, and the structure refined to an R of 12.3%. The carotene chain is nonplanar because of nonbonded interactions. In describing the transition from 11-*cis*-retinal to all-*trans*-retinal, it is necessary to mention not only the rotation of 180° about the C(11)–C(12) double bond, but also a rather unexpected rotation of $141.3 \pm 0.9^\circ$ about the C(12)–C(13) single bond.

Introduction

The carotenoid compound, 11-*cis*-retinal, acts as a photochemical sensor in visual systems. In the dark, 11-*cis*-retinal is covalently linked to proteins in the retina known as opsins. The opsins differ in differing species and may differ in different cells of the retina. The rod cells and the three types of cone cells found in the human retina have distinct opsin molecules, but all have 11-*cis*-retinal as their chromophore. The primary event in the visual process is the photochemical isomerization of the bound 11-*cis*-retinal to all-*trans*-retinal:



Other transformations in the retina follow, but it has been shown that these subsequent events can occur in the dark (Wald, 1968; Hubbard & Kropf, 1958).

Despite the considerable interest in the 11-*cis* and all-*trans* retinals due to their biological function, the details of their geometry had not been determined.

Indeed, even the gross features of the conformation of the *cis* isomer have long been a subject of theoretical discussion (see Hubbard & Wald, 1968). For this reason, an X-ray diffraction analysis was undertaken on the two most important isomers of retinal, the 11-*cis* and the all-*trans* forms. A preliminary report giving an approximate idea of their conformations has been published (Gilardi, Karle, Karle & Sperling, 1971). A careful analysis of the structure of all-*trans*-retinal was independently executed and recently reported (Hamanaka, Mitsui, Ashida & Kakudo, 1972). A brief communication comparing the parameters from the two independent determinations for all-*trans*-retinal will be published separately; the agreement between the two determinations is generally quite close.

As would be expected, the 11-*cis*-retinal molecule is unstable to light, oxygen and temperature. In solution, the *cis* isomer isomerizes to the *trans* form very readily at room temperature. As a consequence special precautions are required for the collection of X-ray diffraction data.

Experimental

The 11-*cis*-retinal was synthesized from commercially available *trans*-retinal by Dr Walter Sperling of the Ohio State University Institute for Research in Vision (Dieterle & Robeson, 1954; Oroshnik, Brown, Hubbard & Wald, 1956). Crystals were grown by Dr Sperling and were transported and stored at dry ice temperatures in ampoules filled with dry nitrogen.

Weissenberg and precession photographs of crystals mounted on the a axis established the space group as $P2_1/c$. All crystals of 11-*cis*-retinal examined were found to be twinned, with the components of the twin present in unequal amounts. The collection of the intensities on an automatic diffractometer was carried out as if there were no minor twin. Corrections for overlap for the minor twin are discussed below.

The major hindrance to accurate data collection was crystal deterioration. The surfaces of the crystals became glassy and rounded when exposed to normal atmosphere and temperature. This deterioration was accompanied by a large drop in some, and eventually all, of the reflection intensities. However, it was soon found that most of the intensity loss was due to a shifting in the alignment of the crystal. The shifting of these crystals is probably linked to the deterioration of the mounting surface of the crystal and was observed with several types of adhesives. The shifting of alignment was even more evident when a crystal was mounted without adhesive in a glass capillary. Although the preliminary experiments showed that significant deterioration did occur, it was apparent that the amount of deterioration was tolerable if the crystal could be kept aligned, and that the major cause of the decay was not irradiation by X-rays.

The X-ray intensity data were collected from a freshly prepared crystal on a specially prepared automatic diffractometer. It was desirable to make the complete data collection from one crystal because the amount of twinning varied from specimen to specimen. The four-circle goniostat was enclosed in a light-tight enclosure continuously purged with dry nitrogen. Thus, the possible deleterious effects of visible light, oxygen, and water vapor were minimized. The room was cooled slightly (16–17°C). The crystal was realigned twice during the data collection. Realignment returned the intensity readings for the reflections used for monitoring the data collection to values exceeding 0.9 of their original values. By the fifth day of data collection, the standards could no longer be returned to these values by realignment and the data collection was discontinued. A total of 2345 independent reflections were obtained, which is 81% of all that would

be normally collected on the diffractometer from a copper target. All of the data collected were corrected to compensate for the decay of three monitored reflections, which were measured after each batch of 30 data measurements. Their intensities fell to 70 to 80% of their original values as the alignment deteriorated.

The mode of data collection was the θ - 2θ scan technique with a scan of $2.2^\circ + 2\theta(\alpha_2)^\circ - 2\theta(\alpha_1)^\circ$ and a background count of 4 sec at either end of the scan. Because of the instability of the orientation of the crystal, the use of scanning was indicated as opposed to peak-height measurements, even though the latter technique would have been much faster. The cell parameters and other physical data are listed in Table 1.

Table 1. *Physical data*

Molecular formula	C ₂₀ H ₂₈ O
Molecular weight	284.45
Habit	Parallelepiped
Dimensions ($a \times b \times c$)	0.23 × 0.14 × 0.10 mm
Space group	$P2_1/c$
Z	4
a	7.540 (4) Å
b	10.666 (6)
c	22.102 (20)
$\cos \beta, \beta$	-0.0904 (11), 95.19 (6)°
Volume	1770 Å ³
Density, calc.	1.07 g.cm. ⁻³
Radiation	Cu K α , Ni-filtered, 1.54178 Å
Linear absorption coeff. μ	4.35 cm. ⁻¹
Temperature	16–17°C
Number of independent reflections observed	2345

The phases associated with the strongest normalized structure factors were determined by using the symbolic addition procedure (Karle & Karle, 1966). Of

Table 2. *Fractional coordinates and thermal parameters with standard deviations*

The thermal parameters are of the form

$$T = \exp[-\frac{1}{4}(B_{11}h^2a^*2 + B_{22}k^2b^*2 + B_{33}l^2c^*2 + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$$

Standard deviations are based solely on least-squares parameters.

	X	Y	Z	A		B		C	
				11	22	33	12	13	23
C(1)	1.1271(7)	0.3589(4)	0.2697(2)	3.5(2)	2.9(2)	4.3(2)	0.2(2)	0.9(2)	-0.8(2)
C(2)	1.2253(10)	0.3826(6)	0.2135(3)	7.5(4)	6.1(3)	4.8(3)	-2.2(3)	2.8(3)	-1.6(2)
C(3)	1.4181(9)	0.4121(7)	0.2294(3)	5.7(4)	7.3(4)	6.5(3)	-1.2(3)	3.5(3)	-2.4(3)
C(4)	1.4430(8)	0.5281(6)	0.2702(3)	4.1(3)	5.4(3)	6.4(3)	-0.6(2)	2.5(2)	-0.7(2)
C(5)	1.3111(6)	0.5346(4)	0.3183(2)	2.7(2)	3.3(2)	4.7(2)	0.3(2)	0.7(2)	0.4(2)
C(6)	1.1692(6)	0.4600(4)	0.3179(2)	2.9(2)	2.7(2)	3.3(2)	0.3(2)	0.7(2)	0.1(1)
C(7)	1.0391(6)	0.4665(4)	0.3645(2)	2.7(2)	2.7(2)	3.8(2)	-0.0(2)	0.3(2)	0.2(2)
C(8)	0.9753(6)	0.5712(4)	0.3880(2)	2.7(2)	2.8(2)	3.4(2)	-0.1(2)	0.7(2)	-0.1(1)
C(9)	0.8467(6)	0.5780(4)	0.4335(2)	2.5(2)	3.0(2)	3.5(2)	0.0(2)	0.4(2)	0.1(1)
C(10)	0.8171(6)	0.6909(4)	0.4580(2)	2.4(2)	3.3(2)	3.8(2)	-0.2(2)	0.7(2)	-0.2(2)
C(11)	0.7036(7)	0.7138(5)	0.5067(2)	3.8(3)	4.0(2)	4.2(2)	0.1(2)	1.0(2)	-0.1(2)
C(12)	0.6677(7)	0.8234(5)	0.5325(2)	3.9(3)	4.3(2)	4.6(2)	0.2(2)	1.5(2)	-0.3(2)
C(13)	0.7262(7)	0.9509(5)	0.5184(2)	3.1(2)	4.1(2)	5.0(2)	1.0(2)	0.8(2)	-1.3(2)
C(14)	0.7391(7)	0.9883(5)	0.4603(2)	4.2(3)	3.1(2)	4.8(2)	1.0(2)	1.1(2)	-0.3(2)
C(15)	0.7964(10)	1.1147(6)	0.4445(3)	7.1(4)	3.8(3)	7.1(4)	0.9(3)	2.3(3)	-0.2(2)
C(16)	1.3680(7)	0.6293(5)	0.3664(3)	3.4(3)	4.5(3)	5.4(3)	-0.8(2)	-0.0(2)	-0.9(2)
C(17)	1.1717(10)	0.2299(5)	0.2970(3)	8.1(4)	3.1(2)	5.9(3)	0.3(3)	1.7(3)	-0.1(2)
C(18)	0.9239(9)	0.3561(6)	0.2482(3)	5.7(3)	6.1(3)	5.9(3)	-1.2(3)	0.2(3)	-2.1(3)
C(19)	0.7607(7)	0.4575(5)	0.4514(2)	4.4(3)	3.1(2)	5.6(3)	-0.4(2)	2.2(2)	0.0(2)
C(20)	0.7611(10)	1.0350(6)	0.5741(3)	7.1(4)	5.7(3)	5.7(3)	0.1(3)	0.9(3)	-2.6(3)
OXY	0.7939(9)	1.1484(5)	0.3920(3)	13.9(5)	5.0(2)	8.1(3)	0.0(3)	5.3(3)	0.3(2)

Table 2 (cont.)

H(1A1)	1.1372	0.1996	0.3399
H(1A2)	1.1661	0.1582	0.2729
H(1A3)	1.3282	0.2323	0.3160
H(1B1)	0.8227	0.3086	0.2765
H(1B2)	0.8907	0.3170	0.2046
H(1B3)	0.8976	0.4319	0.2267
H(2A)	1.2051	0.3055	0.1813
H(3A)	0.4999	0.3314	0.2579
H(3B)	1.4426	0.4058	0.1849
H(4A)	0.5877	0.5434	0.2838
H(4B)	1.3924	0.6026	0.2336
H(5A)	1.4753	0.6802	0.3761
H(5B)	1.3221	0.7191	0.3478
H(5C)	1.3207	0.6046	0.4023
H(7)	0.9841	0.3870	0.3734
H(8)	1.0268	0.6567	0.3761
H(9A)	0.8678	0.3834	0.4631
H(9B)	0.7291	0.4167	0.4040
H(9C)	0.6485	0.4738	0.4772
H(10)	0.9204	0.7615	0.4549
H(11)	0.6606	0.6369	0.5329
H(12)	0.5982	0.8222	0.5771
H(14)	0.6839	0.9283	0.4204
H(15)	0.8187	1.1803	0.4874

the two possibilities corresponding to the values of one unknown symbol, the first *E* map which was calculated showed the 21 nonhydrogen atoms.

The structure was refined using the Busing, Martin & Levy (1962) program for full-matrix least-squares minimization of $\sum w(|F_o| - |F_c|)^2$. The scattering factors listed in *International Tables for X-ray Crystallography* (1962) were used. Three cycles of isotropic refinement brought the *R* value to 0.196. The correction for overlap from a small (5%) twin component changed the *R* value insignificantly to 0.193. Conversion to anisotropic thermal factors and two cycles of refinement led to an *R* of 0.16. A difference map at this stage of the refinement revealed all the hydrogen atoms along the carotenoid chain and most of the aliphatic hydrogens. Inclusion of 24 of the 28 hydrogen atoms and further refinement gave an *R* of 0.123. No data were considered 'unobserved'. The hydrogen atom parameters were held constant in the refinement; the positions were taken from a difference map and the anisotropic thermal factors were taken to be equal to those of the adjacent carbon atom. On the final cycle of anisotropic refinement, the nonhydrogen atom parameters shifted, on the average, 0.14 standard deviations. The maximum shift was 0.38 σ . During the final cycles of anisotropic refinement, weights were

assigned as follows: $w^{-1} = (|F_o|/10)^2$, $|F_o| \geq 10$; $w = 1$, $0 < |F_o| < 10$; $w^{-1} = \frac{1}{2}$, $F_o = 0$. The weighting scheme cannot, of course, compensate for the systematic errors associated with the deterioration of crystal intensity and alignment which could not be completely removed by the data correction described earlier. In spite of the relatively large systematic errors, the thermal factors show a rather normal trend, namely, smaller values in the middle of the molecule and larger values at the ends. Table 2 gives the fractional atomic coordinates and thermal parameters, and Table 3, the observed and calculated structure factors.

Discussion

The molecular structure of 11-*cis*-retinal is illustrated in a stereodrawing, Fig. 1. The bond distances, angles, and atom numbering scheme are presented in Fig. 2. The molecule contains three planar segments. The atoms in the carotenoid chain from C(6) to C(13) lie within ± 0.08 Å of a plane; the atoms from C(12) to the end of the chain are within ± 0.035 Å of a second plane; and the atoms C(1), C(4), C(5), C(6), C(7), and C(16) are all within ± 0.05 Å of a third plane. The dihedral angle between the first and second planes measured by the rotation about the C(12)–C(13) bond is 38.7° and the dihedral angle between the first and third planes measured by the rotation about the C(6)–C(7) bond is 41.4°. The conformation about the single bonds C(6)–C(7) and C(12)–C(13) is designated as *s-cis*.* In the past, the structural formula of 11-*cis* retinal had generally been drawn with an *s-trans* arrangement at the C(12)–C(13) bond. All of the single bonds of the chain are *s-trans* in the all-*trans* isomer of retinal, and thus the isomerization process must consist of a 140° rotation about the single bond C(12)–C(13), as well as the 180° rotation about the double bond C(11)–C(12).

The 11-*cis*-retinal molecule has been extensively studied theoretically. Nash (1968) and Pullman, Langlet & Berthod (1969) found low-energy conforma-

* The prefix *s* before *cis* or *trans* indicates that a *single* bond is referred to; *s-cis* and *s-trans*, used in the carotenoid literature, roughly correspond to the terms *syn* and *anti* used in conformational analysis (cf. Klyne & Prelog, 1960).

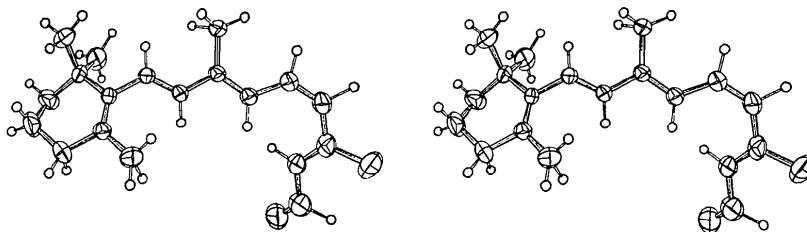


Fig. 1. A stereodrawing of the 11-*cis*-retinal molecule. The hydrogen atoms on the methyl carbon at the far right in the illustration, C(20), are not shown because they were not prominent in difference maps; all of the hydrogen atoms shown were found in a map. Figs. 1, 3, and 4 were drawn with the aid of the ORTEP computer program of Johnson (1965).

Table 3. Observed and calculated structure factors for 11-cis retinal. The columns are the index l, |Fo| x 10, and Fc x 10.

Table with columns for index l, |Fo| x 10, and Fc x 10. The table contains multiple rows of numerical data representing structure factors for different indices.

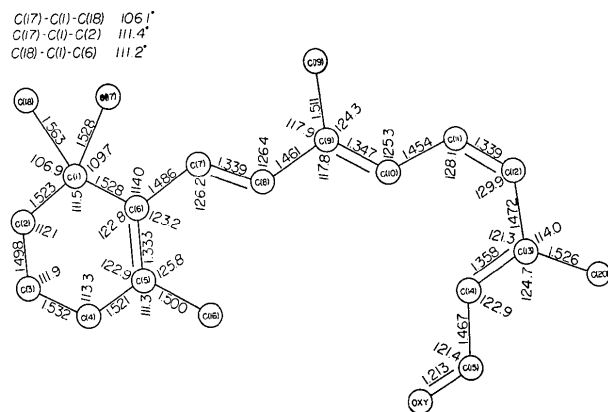


Fig. 2. Bond lengths, angles, and the numbering scheme for 11-*cis* retinal. Estimated standard deviations for the bond distances range from 0.006 to 0.010 Å, and for the bond angles are from 0.5 to 0.75°.

tions which had large (40–50°) torsions about the C(10)–C(11) single bond. This bond is quite planar in the crystal (see Table 4). Also, both studies concluded that C(12)–C(13) torsion would be close to *s-trans*. Rotation about the double bond C(11)–C(12) was considered and large torsions ($\varphi > 10^\circ$) were considered unlikely. However, molecular orbital calculations were interpreted by Wiesenfeld & Abrahamson (1968) as indicating that rotation about the double bond (11–12) would be more effective, overall, than any single bond torsion. In the crystal, the double bonds of 11-*cis*-retinal show very small torsions (see Table 4).

The n.m.r. spectrum of 11-*cis*-retinal was correctly interpreted by Patel (1969) to imply that the C(10)–C(11) single bond should be close to *s-trans* planar and that the steric hindrance would be relieved by rotation about the C(12)–C(13) bond. These predictions were corroborated by this crystal structure analysis. The recent molecular orbital calculations of Honig & Karplus (1971) predict the configuration of 11-*cis*-retinal to be essentially the same as that found in the crystal. The excellent agreement between the diffraction results, the m.o. calculations, and the n.m.r. inter-

pretation, which is based on observations of retinal molecules in solution, indicate that the form found in the crystal may indeed predominate in solution.

Table 4. Torsion angles, 11-*cis*-retinal

The numbers in parentheses are the estimated standard deviations in the last significant figure. The torsion angle e.s.d.'s were derived from the e.s.d.'s of the positional parameters of the four atoms obtained from the least-squares refinement, assuming the errors in the positions to be independent (uncorrelated). The torsion angle is defined in Klyne & Prelog (1960).

Single bonds	1-2-3-4	-59.1 (7)°
	2-3-4-5	39.8 (7)
	3-4-5-6	-11.5 (8)
	5-6-1-2	-18.8 (7)
	6-1-2-3	47.4 (7)
	5-6-7-8	41.4 (7)
	7-8-9-10	170.8 (5)
	9-10-11-12	-179.3 (5)
	11-12-13-14	38.7 (9)
	13-14-15-Oxy	-174.5 (7)
	7-8-9-19	-7.2 (7)
	7-6-1-18	42.8 (6)
	7-6-1-17	-74.3 (5)
Double bonds	4-5-6-1	1.1 (7)°
	16-5-6-7	3.9 (8)
	6-7-8-9	179.5 (4)
	8-9-10-11	-175.5 (4)
	10-11-12-13	2.1 (9)
	12-13-14-15	179.8 (5)
	20-13-14-15	3.1 (9)

The relief of steric crowding is facilitated by slight distortions in bond angles. Wherever a methyl group is attached to a carotenoid chain, the angle opposite the methyl group is less than the expected value ($\sim 125^\circ$); e.g. the average value of the C(8)–C(9)–C(10) angle in several all-*trans* carotenoids is 117.5° (Bart & MacGillavry, 1968a). This angle is 117.7° in 11-*cis*-retinal. The comparable angle C(12)–C(13)–C(14) is slightly larger, 120.6°, owing perhaps to the crowding in the vicinity of C(11) and C(12). The angles at atoms C(11) and C(12) are somewhat larger than average, 127.3 and 130.3° respectively. This may also result from the crowding. Short intramolecular distances between nonbonded atoms in 11-*cis*-retinal are listed

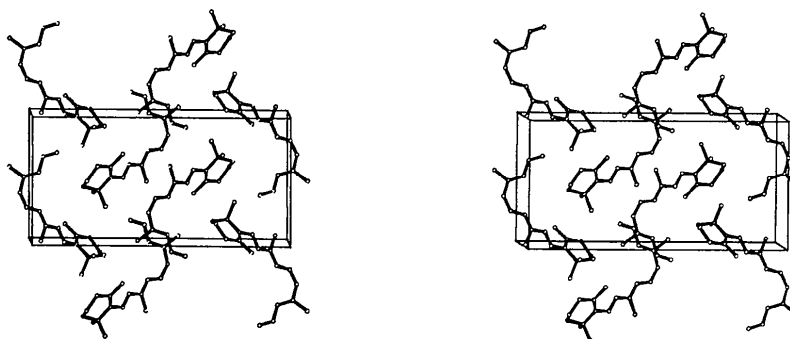


Fig. 3. A stereo packing diagram. The unit cell is viewed down the *a* axis, with *b* vertical and *c** horizontal.

in Table 5, along with corresponding distances in all-*trans*-retinal. It can be seen that the contacts C(10)···C(13) and C(10)···C(14) are no shorter than the methyl···chain atom contacts found in all-*trans* carotenoids. One of these contacts, C(11)···C(20), is only 2.93 Å in all-*trans* retinal and is *relieved* by the *trans-cis* isomerization.

Table 5. Short intramolecular distances in 11-*cis*- and all-*trans*-retinal

Distances enclosed in parentheses are not short, but are included for comparison with short contacts present in the other isomer.

	11- <i>cis</i> -retinal	all- <i>trans</i> -retinal
C(7)···C(16)	3.024 Å	2.977 Å
C(7)···C(17)	3.141	3.115
C(7)···C(18)	2.888	2.945
C(7)···C(19)	2.977	2.955
C(8)···C(5)	3.104	3.123
C(8)···C(16)	3.104	3.195
C(10)···C(13)	3.180	(3.841)
C(10)···C(14)	3.228	(4.850)
C(11)···C(14)	3.122	(3.666)
C(11)···C(19)	3.039	3.049
C(11)···C(20)	(3.746)	2.925
C(15)···C(20)	3.022	3.050

One of the keys to the molecular stability of 11-*cis*-retinal is the continuance of electronic conjugation along the chain despite the large torsion at C(12)–C(13). The average single bond length within *trans* carotenoid chains is ~ 1.45 Å, with the single bond adjacent to the ring 0.02–0.03 Å longer (Bart & MacGillavry, 1968a).

Single bonds in 11-*cis* retinal are only $2\text{--}3\sigma$ longer than this average (*i.e.*, 1.47–1.48 Å). The single bond C(12)–C(13) is not noticeably lengthened by the disruption of planarity at that point in the chain. Double bonds are within the normal carotenoid range. The single bond between C(2) and C(3) of the cyclohexene ring is short, hinting at the possibility of a puckering disorder, such as was more clearly indicated in all-*trans*-retinal (Hamanaka *et al.*, 1972) and 15,15'-dehydrocanthaxanthin (Bart & MacGillavry, 1968b).

The *s-cis* attachment of the cyclohexene ring has already been noted in the structures of several carotenoids (see Bart & MacGillavry, 1968a). A molecular orbital calculation of molecular energy *versus* ring-chain torsion (Pullman *et al.*, 1969), indicates a minimum at a torsion of 40° from a planar *s-cis* arrangement, in excellent agreement with these results. There are somewhat less favorable conformational minima predicted to be near the *s-trans* arrangement, and there are two carotenoids whose structures are reported to have the *s-trans* ring inclination, namely the all-*trans* isomer of β -ionylidene crotonic acid (Eichhorn & MacGillavry, 1959) and the five-membered ring analogue of vitamin A acid (Schenk, 1971).

The packing of 11-*cis* retinal is illustrated in Figs. 3 and 4. There are no carbon–carbon or carbon–oxygen contacts less than 3.6 Å. Packing seems to be primarily influenced by van der Waals interactions between hydrogen atoms. There are a few H···H contacts in the 2.1–2.2 Å range, and many at slightly longer distances (2.2–2.4 Å).

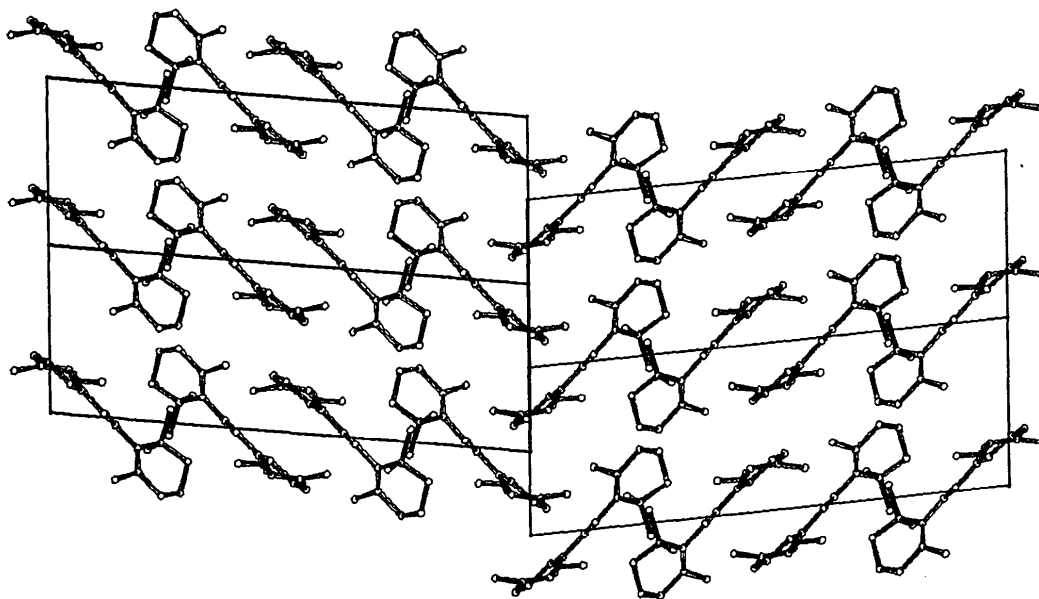


Fig. 4. A packing diagram which shows the relative orientation of the unit cells of the two twin individuals. Unit cells on the right are related to those on the left by a twofold rotation about c^* , followed by a translation of $c + (a/2)$. The cells are oriented so that c^* is horizontal for both sets of cells, while b points *out of* the paper for the cells at the left, and *into* the paper for the cells at the right.

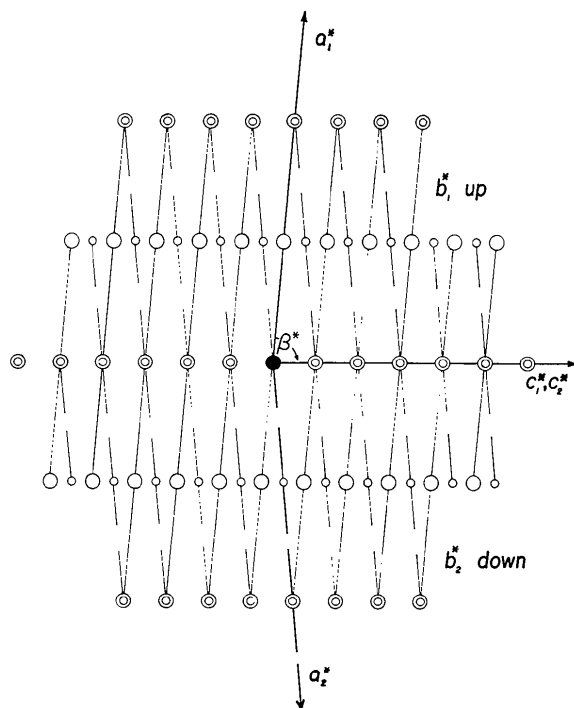


Fig. 5. The superimposed a^*c^* nets of twinned 11-*cis*-retinal. Small circles refer to a minor component and large ones to the major component. On half the layers, the small and large circles overlap.

Twining

The crystals of 11-*cis* retinal were twinned on (001), but the effective diffracting volumes of the individual components were unequal. This facilitated the choice of space group without ambiguity since the contributions of the two $P2_1/c$ lattices were quite apparent in the precession photographs. As shown in Fig. 5, layers with h odd are composed of reflections which do not overlap and layers with h even are composed of reflections which do overlap. An ideal interpretation of the pattern illustrated in Fig. 5 implies a geometric relation which can be expressed in terms of parameters of the real cell:

$$a/2 + 2c \cos \beta = 0. \quad (1)$$

From the measured cell parameters and their estimated standard deviations, an experimental measurement of this quantity is found to be

$$a/2 + 2c \cos \beta = 0.24 \pm 0.05. \quad (2)$$

The constraint is not obeyed exactly; there is not a perfect coincidence of spots on layers of even h . However, calculation of the diffractometer settings for each of the components indicated that with this small variation from the ideal, the reflections would not be resolved.

The overlapping reflections do not, in general, have the same or symmetry-equivalent indices. The $(hkl)_1$

reflection overlaps the $(\bar{h}\bar{k}l + h/2)_2$ reflection, where the subscript refers to the diffracting component. Since one component of the twin dominated in the diffraction, the first treatment of the data neglected overlap entirely. This approximation did not affect the solution of the structure, *i.e.* the phasing of the structure factors. However, it was recognized that the contribution of each individual contributor to the composite reflections could be extracted from the available data.

Assuming that the components are identical in structure, and differ only in effective volume by a constant scale factor,

$$I_2(hkl) = KI_1(hkl). \quad (3)$$

As indicated above, the composite reflections are made up of contributions with differing indices:

$$I_{\text{obs}}(hkl) = I_1(hkl) + I_2(\bar{h}\bar{k}l + h/2). \quad (4)$$

Using (3), this can also be written:

$$I_{\text{obs}}(hkl) = I_1(hkl) + KI_1(\bar{h}\bar{k}l + h/2). \quad (5)$$

Similarly, one can write

$$I_{\text{obs}}(\bar{h}\bar{k}l + h/2) = I_1(\bar{h}\bar{k}l + h/2) + KI_1(hkl). \quad (6)$$

Equations (5) and (6) can be solved to yield the unknown $I_1(hkl)$:

$$I_1(hkl) = \frac{I_{\text{obs}}(hkl) - KI_{\text{obs}}(\bar{h}\bar{k}l + h/2)}{(1 - K^2)}. \quad (7)$$

The constant, K , can be measured by collecting a limited amount of data from layers of odd h from each individual. For the crystal used for data collection, the factor K was 0.05. Using this value for K and equation (7), the data were corrected for overlap after the isotropic least-squares refinement had converged to an R index of 0.196. After correction, the parameters were again refined isotropically, and the R index converged to 0.193. The anisotropic refinement was carried out only on the corrected data.

The relative orientations of the unit cells of the two contributors to the twin are depicted in Fig. 4. One of the possible ways of fitting the two lattices together is shown, namely, arranging the lattices so as to share the (001) plane after a shift along a of $a/2$. However, calculation of distances across the interface reveals 9 intermolecular distances that are less than normal van der Waals contacts, ranging from 2.87 to 3.4 Å.

The lower values are not acceptable and some adjustment in the attachment of the two components would be required to increase the contacts by a few tenths of an Ångstrom unit.

References

- BART, J. C. J. & MACGILLAVRY, C. H. (1968a). *Acta Cryst.* B24, 1587.
 BART, J. C. J. & MACGILLAVRY, C. H. (1968b). *Acta Cryst.* B24, 1569.

- BUSING, W. R., MARTIN, K. O. & LEVY, H. A. (1962). *ORFLS*. Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- DIETERLE, J. M. & ROBESON, C. D. (1954). *Science*, **120**, 219.
- EICHHORN, E. L. & MACGILLAVRY, C. H. (1959). *Acta Cryst.* **12**, 872.
- GILARDI, R. D., KARLE, I. L., KARLE, J. & SPERLING, W. (1971). *Nature, Lond.* **232**, 187.
- HAMANAKA, T., MITSUI, T., ASHIDA, T. & KAKUDO, M. (1972). *Acta Cryst.* **B28**, 214.
- HONIG, B. & KARPLUS, M. (1971). *Nature, Lond.* **229**, 558.
- HUBBARD, R. & KROPF, A. (1958). *Proc. Natl. Acad. Sci. U.S.A.* **44**, 130.
- HUBBARD, R. & WALD, G. (1968). *Structural Chemistry and Molecular Biology*, p. 545. Edited by A. RICH & N. DAVIDSON. San Francisco: Freeman.
- International Tables for X-ray Crystallography* (1962). Vol. III. Birmingham: Kynoch Press.
- JOHNSON, C. K. (1965). *ORTEP*. Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
- KARLE, J. & KARLE, I. L. (1966). *Acta Cryst.* **21**, 849.
- KLYNE, W. & PRELOG, V. (1960). *Experientia*, **16**, 521.
- NASH, H. A. (1969). *J. Theor. Biol.* **22**, 314.
- OROSHNIK, W., BROWN, P. K., HUBBARD, R. & WALD, G. (1956). *Proc. Natl. Acad. Sci. U.S.A.* **42**, 578.
- PATEL, D. J. (1969). *Nature, Lond.* **221**, 825.
- PULLMAN, B., LANGLET, J. & BERTHOD, H. (1969). *J. Theor. Biol.* **23**, 492.
- SCHENK, H. (1971). *Acta Cryst.* **B27**, 667.
- WALD, G. (1968). *Nature, Lond.* **219**, 800.
- WIESENFELD, J. R. & ABRAHAMSON, E. W. (1968). *Photochem. Photobiol.* **8**, 487.

Acta Cryst. (1972). **B28**, 2612

The Crystal and Molecular Structure of Sulphur-Containing Heterocyclic Ring Compounds. III. 3-Methyl-4-oxo-1,3-thiazine-2-thione

BY V. AMIRTHALINGAM AND V. S. JAKKAL

Chemistry Division, Bhabha Atomic Research Centre, Trombay, Bombay-85, India

(Received 25 April 1972)

3-Methyl-4-oxo-1,3-thiazine-2-thione, $C_5H_7NOS_2$, is monoclinic, space group $P2_1/c$, $Z=4$. The structure was solved by Patterson and minimum function methods with 623 visually estimated X-ray intensities, and refined to $R=0.108$. The six-membered ring has a boat conformation with the outer sulphur atom at the 2-position *trans* to the methyl group and to the methylene carbon atom at the 6-position. These latter two groups are *cis* to one another.

Introduction

3-Methyl-4-oxo-1,3-thiazine-2-thione is the third in a series of sulphur-containing heterocyclic compounds to be subjected to X-ray structure analysis in this department. Of interest is the state of conjugation in the six-membered ring, and also its geometry and conformation in comparison with similar planar five-membered rings whose structures have been reported (Amirthalingam & Muralidharan, 1972*a, b*).

Experimental

The yellow needles are monoclinic. The unit-cell parameters derived from Weissenberg photographs taken with Cu $K\alpha$ radiation ($\lambda=1.542 \text{ \AA}$) are $a=8.02$ (1), $b=7.11$ (1), $c=15.47$ (2) \AA , $\beta=124$ (1) $^\circ$. There are four molecules in the cell ($d_o=1.45$, $d_c=1.47 \text{ g.cm}^{-3}$). The systematic absences $h0l$ for l odd and $0k0$ for k odd characterize the space group as $P2_1/c$. The reflexions $h0l$ to $h6l$, and $hk0$ were recorded with Cu $K\alpha$ radiation using the equi-inclination Weissenberg method, and their intensities were estimated visually. 623 unique

reflexions were measured and corrected for Lorentz, polarization, and spot-size effects, but not for absorption. Scaling was achieved by using Wilson plots.

A three-dimensional Patterson synthesis was computed and the peaks near the origin as well as the Harker peaks were examined. It was possible to identify the peaks due to intramolecular non-bonded S...S atoms, and also those from symmetry related S atoms. Minimum function methods revealed the image of the six-membered ring, and iterative Fourier methods located the remaining atoms. The structure was refined by full-matrix least-squares calculations. In the final stage of the refinement, a Cruickshank weighting scheme with $w=(2.0+F_o+0.053F_o^2)^{-1}$ was used. The S atoms, the O atom and the methyl C atom were allowed to vibrate anisotropically. The final value of R for all observed reflexions was 0.108. Hydrogen atoms were ignored. Form factors for neutral atoms were taken from *International Tables for X-ray Crystallography* (1962). The structure projected down b is shown in Fig. 1. The final parameters are given in Table 1. Table 2 lists the values of F_o and F_c .