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Isomers, Visual Pigment, and Bacteriorhodopsin Analogs of 3,7,13-Trimethyl-10-isopropyl-2,4,6,8,10-tetradecapentaenal and 3,7,11-Trimethyl-10-isopropyl-2,4,6,8,10-dodecapentaenal (Two Ring Open Retinal Analogs)[1]

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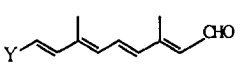
Six geometric isomers of **2** (3,7,13-trimethyl-10-isopropyl-2,4,6,8,10-tetradecapentaenal) have been isolated and characterized. Of these, four (equivalent to 9-*cis*, 11-*cis*, 5,9-*dicis*, and 5,11-*dicis* of retinoids) were found to form stable visual pigment analogs. So were four isomers (equivalent to 7-*cis*, 9-*cis*, 11-*cis*, and 9,13-*dicis*) of **3** (3,7,11-trimethyl-10-isopropyl-2,4,6,8,10-dodecapentaenal). These analogs suggest that the hydrophobic pocket is more flexible than what was recognized earlier, perhaps capable of accepting analogs with 6-*S*-*trans* as well as a 6-*S*-*cis* conformers. The presence of bacteriorhodopsin analogs from isomers of **2** was also reported.

INTRODUCTION

Modified retinal analogs have been used extensively to probe for structural information of the binding site of rhodopsin (*1*). Among these are ring open retinal analogs. Their ability to form pigment analogs was demonstrated by Crouch and Or (*2*) and by Kropf (*3*) through the use of compounds such as **1a–1c.** More recently from this laboratory isomers of the related pentaenal, **1d,** were shown to form pigment analogs (*4*). These results are in agreement with the notion of the presence of a hydrophobic pocket in the binding site as demonstrated through inhi-



1

a: Y = 

d: Y = 

b: Y = 3-pentyl
c: Y = 2-butyl
e: Y = isopropyl

bition studies (*5*) which can accept end groups equivalent to or smaller than the trimethylcyclohexenyl ring of the parent retinal (*6*). On the other hand, **1e** (*2*) did not a give a pigment. Because of potential interest in the use of ring open retinal analogs for introduction of reporting groups such as photo-affinity labels (*7*), we

[1] While the proper IUPAC names are given in the title, for ready comparison with retinal, a numbering system for the latter is used for compounds **2** and **3** throughout the text.

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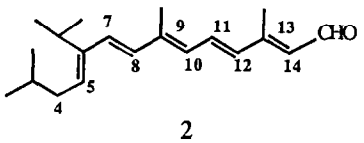
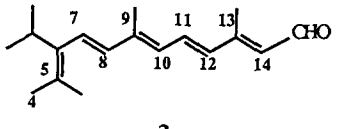
have made two analogs (**2,3**) including one with a bulkier isobutyl substituent. Their interactions with bovine opsin are presented below.



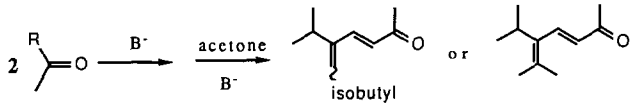
2



3

EXPERIMENTAL

Retinal analogs. For compounds **2** and **3** the corresponding β-ionone analogs were synthesized via successive aldol condensation reactions. Subsequent C_2 and C_5 chain extension reactions (*8*) led to mixtures of isomers of **2** and **3.**



R = isopropyl or isobutyl

For compound **2** HPLC analysis of the synthetic mixture showed the presence of five major peaks. The first peak was that of an inseparable mixture of isomers containing the 13-*cis* geometry. The next four peaks were those of the 5,9-*dicis*, 9-*cis*, 5-*cis*, and all-*trans* isomers in the order of retention times. The assignment of the geometry around the double bonds from C-7 through C-14 followed the well established ^{1}H NMR arguments (*9*) for the parent retinal isomers. The corresponding chemical shift and coupling constant data are listed in Table 1. Assignments of the configuration around 5,6-double bonds are less routine. Only after extensive nuclear Overhauser effect (NOE)[3] experiments were we able to assign with confidence this configuration for all four isomers. The key observations are the following. For 5-*trans* compounds, irradiation of H-8 led to enhancement of signals due to H-5 and the methine hydrogen of the isopropyl group while for 5-*cis* compounds, similar irradiation resulted in enhancement of the methine hydrogen of the isopropyl group and the methylene hydrogens of the isobutyl group.

The 11-*cis* isomers were obtained after photo-irradiation (*9*) of the all-*trans* and the 5-*cis* isomers. The 5-*cis* (Z) configuration was found to be unaffected during irradiation, thus giving three *dicis* isomers (isomerizing at the 9, 11, or 13 position) while the 5-*trans* (E) geometry isomerized competitively (albeit at a lower efficiency) against the 9-, 11-, or 13-double bonds of the polyene chain, giving a mixture of 5-*cis* and 5-*trans* isomers. The two 11-*cis* isomers were isolated by preparative HPLC. The smaller coupling constants (Table 1) revealed the *cis* configuration.

Ultraviolet–visible absorption spectra are characterized by the presence of

[3] Abbreviations used: NOE, nuclear Overhauser effect.

TABLE 1

^{1}H NMR Data of Isomers of Ring-Opened Retinal Analogs **2** and **3**[a]

Compound	Chemical shift (ppm)								Coupling constant (Hz)		
	H_5	H_7	H_8	H_{10}	H_{11}	H_{12}	H_{14}	H_{15}	$J_{7,8}$	$J_{10,11}$	$J_{11,12}$
all-*trans*-**2**	5.64	6.34	6.50	6.25	7.15	6.39	5.99	10.13	15.9	11.3	14.2
13-*cis*-**2**	5.64	6.34	6.51	6.29	7.04	7.30	5.86	10.23	15.8	11.8	14.8
11-*cis*-**2**	5.64	6.34	6.48	6.59	6.70	5.93	6.11	10.12	15.9	12.6	11.3
9-*cis*-**2**	5.68	6.36	6.99	6.10	7.30	6.32	5.99	10.13	15.7	11.6	15.0
5-*cis*-**2**	5.53	6.41	6.74	6.29	7.16	6.40	6.00	10.13	16.1	11.5	15.0
5,13-*dicis*-**2**	5.53	6.43	6.74	6.33	7.06	7.32	5.86	10.23	16.1	11.6	15.1
5,11-*dicis*-**2**	5.53	6.40	6.75	6.63	6.72	5.95	6.11	10.12	16.1	12.5	11.2
5,9-*dicis*-**2**	5.58	6.73	6.92	6.15	7.28	6.34	6.00	10.13	16.0	11.6	15.0
all-*trans*-**3**	—	6.40	6.23	6.20	7.14	6.37	5.97	10.10	16.1	11.3	15.0
13-*cis*-**3**	—	6.40	6.24	6.24	7.03	7.28	5.84	10.20	16.3	11.3	14.8
11-*cis*-**3**[b]	—	6.29	6.16	6.52	6.63	5.93	5.92	10.01	16.1	12.6	10.8
9-*cis*-**3**	—	6.36	6.73	6.09	7.22	6.30	5.97	10.10	16.0	11.6	15.0

[a] QE-300. In $CDCl_3$ except those noted otherwise.
[b] In acetone-d_6 (20%) and CCl_4.

structured main band for the 5E isomers and the lack of fine structures for the 5Z isomers (Fig. 1 and data in Table 2).

For compound **3**, their isomers were isolated (retention times paralleled to those of retinal isomers) and characterized in a similar manner. The absence of isomerism around the 5,6-double bond made structural assignments relatively routine. The spectral data are listed in Tables 1 and 2.

TABLE 2

Absorption Maxima of Visual Pigment Analogs from Isomers of Compounds **2** and **3**

Compound	Retinal	Pigment
	λ_{max}[a]	λ_{max} (yield)[b]
11-*cis*-**2**	371 nm	495 nm (+++)
5,11-*dicis*-**2**	375 nm	505 nm (+++)
9-*cis*-**2**	366 nm	485 nm (+++)
5,9-*dicis*-**2**	370 nm	493 nm (++)
11-*cis*-**3**	369 nm	496 nm (+++)
9-*cis*-**3**	362 nm	481 nm (++)
9,13-*dicis*-**3**[c]	364 nm	473 nm (+++)

[a] In hexane.
[b] In 2% digitonin. +++ ≡ >70%; ++ ≡ 30–69%; + ≡ 3–29%.
[c] Sample containing a small amount of the nonbinding 13-*cis* isomer.

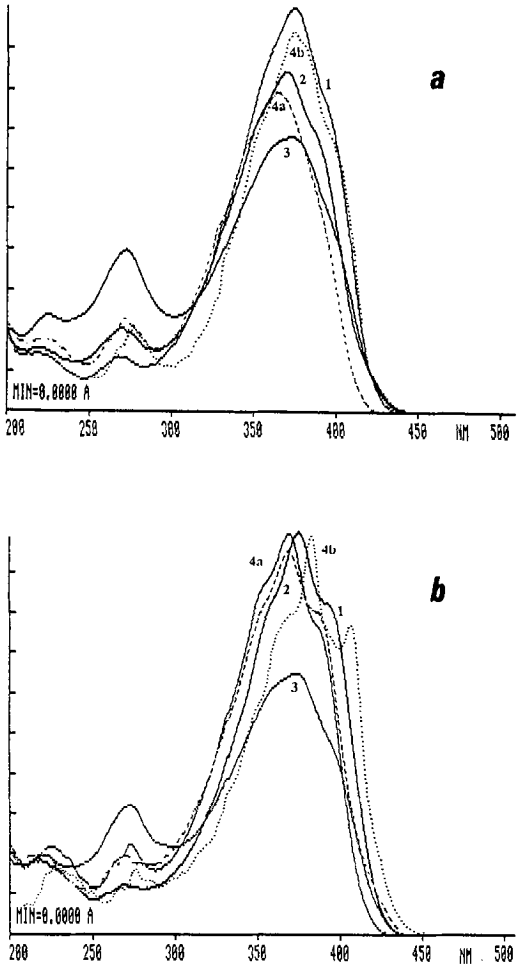


Fig. 1. (a) Ultraviolet–visible absorption spectra of all-*trans* (curve 1), 13-*cis* (2), 11-*cis* (3), and 9-*cis* (4a) of **2**, all in hexane at room temperature. Extinction coefficients for the four isomers are 5.0×10^4, 4.3×10^4, 3.6×10^4 and 4.3×10^4, respectively. Curve 4b is that of the 9-*cis* isomer at 77 K in 3-methylpentane. (b) Ultraviolet–visible absorption spectra of 5-*cis* (curve 1), 5,13-*dicis* (2), 5,11-*dicis* (3), and 5,9-*dicis* (4a) of **2** in hexane at room temperature. Extinction coefficients for the four isomers are 5.3×10^4, 5.1×10^4, 4.3×10^4 and 5.3×10^4, respectively. Curve 4b is that of the 5,9-*dicis* isomer at 77 K in 3-methylpentane.

Methods. NMR experiments including NOE studies were carried out on a Nicolet NM-300 spectrometer. Ultraviolet–visible absorption spectra on a PE-Coleman-120 or a P.E.-λ5 spectrometer. An Oxford Instrument Cryostat (Model 28009A) was used for recording low temperature uv–visible absorption spectra. Binding interactions of the isolated analog isomers with bovine opsin were carried out in the same manner as reported (*10*). Both pairs of the 9-*cis* and 11-*cis* isomers of **2** and several isomers of **3** were found to form stable pigment analogs as

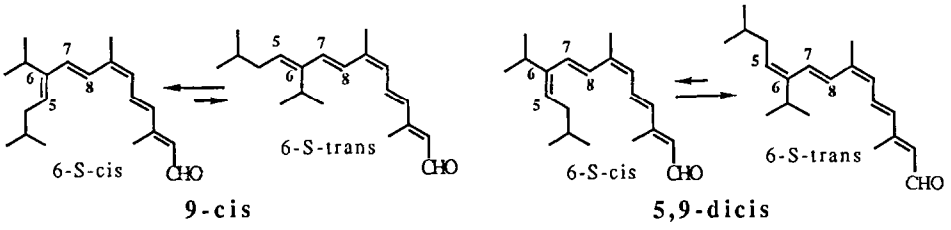
indicated by the typically structureless band centered at 470–505 nm. Pigment yields and absorption maxima are also listed in Table 2. Methods of preparation of bacteriorhodopsin and binding interaction with the retinal analogs are essentially those in the literature (*11*).

RESULTS AND DISCUSSION

Compound **3** does not contain atoms occupying space beyond those in retinal. It is only one or two atoms larger than the active analogs (**1a–1c**) reported by Crouch and Or (*2*). Therefore, the ability of its isomers to interact with bovine opsin is not surprising. On the other hand, isomers of **2,** which have a bulkier isobutyl side chain, are also capable of forming pigment analogs in high yield. The results are consistent with the view of a relatively flexible hydrophobic pocket in opsin (*12*). This point is amplified below.

The presence of 5,6-isomerism and **2** added an extra set of 5-*cis* isomers. Binding studies show that opsin reacted indiscriminately, perhaps unexpectedly, with both sets of isomers (9-*cis*, 11-*cis* versus, 5,9-*dicis*, 5,11-*dicis*) giving pigments in high yields. The absorption maxima (Table 2) are different within each pair of 5-*trans*/5-*cis* isomers with the 5-*cis* being more red-shifted, and values bracketing those from retinal.

In an attempt to understand the effect of molecular structures on binding interaction and absorption characteristics, we carried out conformational analysis around the 6,7-bond for the 9-*cis* and the 5,9-*dicis* isomers, a representative pair of 5-*cis*/5-*trans* isomers in this series. MMP2(85)[4] calculations showed that for 9-*cis*-**2,** the 6-*S*-*cis* conformer is more stable than the 6-*S*-*trans* conformer by 1.6 kcal/mol while for the 5,9-*dicis* isomer the 6-*S*-*trans* conformer is more stable by 1.2 kcal/mol. Hence, the 9-*cis* isomer should exist primarily in the 6-*S*-*cis* form while the 5,9-*dicis* is in the 6-*S*-*trans* form. Several experimental observations appear to



be in agreement with this calculated result. For example, the NOE experiment of 9-*cis*-**2** showed that irradiation of H-8 led to a larger enhancement of H-5 than the methine hydrogen of the isopropyl group while for the 5,9-*dicis* isomer a similar irradiation caused a larger enhancement of the same methine hydrogen than the methylene hydrogens of the isobutyl group. The fine structures in the main band of the uv–visible absorption spectra of the 5-*cis* isomers are probably due to the

[4] An updated version of MMP2: see Allinger, N. L., Q.C.P.E., 1985. Obtained from QCPE, Department of Chemistry, University of Indiana, Bloomington, IN 47405.

more planar *S-trans* conformation and the absence of such a feature in the corresponding 5-*trans* isomer due to the twisted *S-cis* conformation. These features persisted at low temperatures. At 77 K the uv–visible absorption spectra of the 5,9-*dicis* isomer showed enhanced fine structures (Fig. 1) and a concomitant red shift of the absorption band while the spectrum of the 9-*cis* isomer remained structureless at this temperature.

The above analysis suggests that binding interaction of the 5-*cis* isomers may involve the 6-*S-trans* conformer, the form present in higher abundance. For the 6-*S-cis* conformer, it will have the additional disadvantage in having much of the isobutyl group projecting beyond the space occupied by the 5-methyl group of the parent retinyl chromophore. The observed red shifts for the 5-*cis* pigments (5,9-*dicis* from 9-*cis* and 5,11-*dicis* from 11-*cis*) cannot readily be attributed to the *S-trans* conformation. In fact, an *S-cis* linkage in a conjugated chromophore usually causes a red shift (+39 nm for homoannular dienes (*13*)). It is possible that the degree of planarity in 6-*S-cis* and 6-*S-trans* chromophores in a protein environment are different. Any variation of the extent of secondary interaction with the protein could also have a significant effect on its absorption spectra.

The implication that 6-*S-trans* conformer can also fit into the binding site deviates from the commonly accepted notion of the preferred 6-*S-cis* conformation for the retinyl chromophore in rhodopsin (*1*). However, the difference could simply reflect the lack of selectivity of opsin by accepting the more dominant 6-*S-cis* conformer in the parent retinal and the corresponding conformers in the present isomers. It will be of interest to examine the bound chromophore conformation by spectroscopic techniques.

We have carried out preliminary binding experiments of all-*trans* and 5-*cis* isomers of **2** with bacterioopsin. Interestingly, both isomers gave pigments immediately after mixing with bacterioopsin. From all-*trans*-**2,** the absorption maximum of the pigment (dark adapted) was found to be at 478.7 nm. The same absorption maximum was attained when a sample of 13-*cis*-**2** was used instead. For 5-*cis*-**2,** the pigment absorption maximum was found to be at 480.4 nm, and a pigment with identical absorption maximum was obtained when starting with 5-*cis*,13-*cis*-**2.** Hence, while the 13-*cis*/*trans* isomers interconverted expectedly during dark adaptation, the 5-*cis*/5-*trans* pigments apparently did not. And, again the 5-*cis* pigments exhibited a slight red shift from the 5-*trans*. The rates of pigment formation of all-*trans*-**2** and 5-*cis*-**2** with bacterioopsin were very similar. Since these two isomers are believed to exist in different conformers (see above) these qualitative observations are consistent with the view of a nonselective hydrophobic pocket in bacteriorhodopsin with the retinyl chromophore existing in either the 6-*S-cis* or the 6-*S-trans* form (*14*).

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