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Communication

9,11-Di-*cis*-retinal and 9,11-Di-*cis*-rhodopsin[1]

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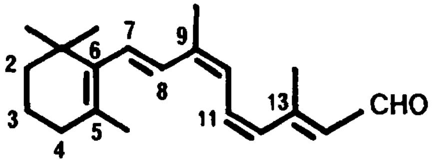
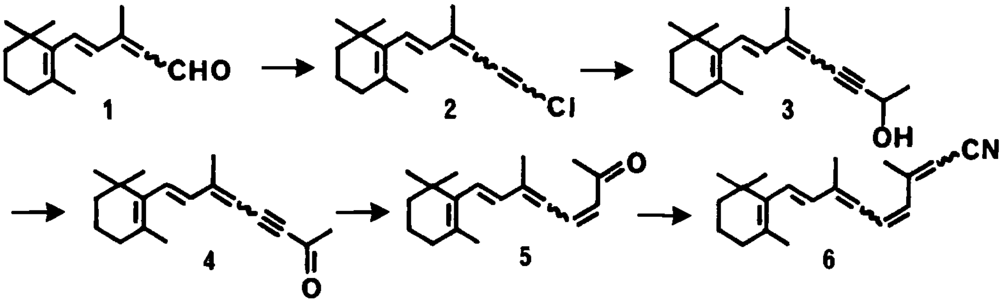
Pursuing the current interest in studies of visual pigment analogs, we wish to report here the synthesis and identification of a new geometric isomer of retinal: 9,11-di-*cis*-retinal and results of its interaction with cattle opsin. It is the 12th known geometric isomer of vitamin A[3] out of the possible 16.



The method of preparation of the new isomer parallels the sequence of reactions we followed recently in the synthesis of 7,11-di-*cis*-retinal (*1*). The synthetic sequence is shown in the following scheme:



A mixture of the isomeric C_{15}-aldehydes was allowed to react with the chloromethyl Wittig reagent to give the tetraene **2**. Base-catalyzed elimination, reaction with acetaldehyde, followed by reaction with manganese dioxide gave the C_{18}-ketone 4. The 11-*cis* geometry was cleanly introduced by hydrogenation of the ketone over Lindlar catalyst. Reaction with the C_2-phosphonate under conditions

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[1] New geometric isomers of vitamin A and carotenoids (8).

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[3] The 11 known isomers are: all-*trans*, 7-*cis*, 9-*cis*, 11-*cis*, 13-*cis*, 7,9-di-*cis*, 7,11-di-*cis*, 7,13-di-*cis*, 9,13-di-*cis*, 11,13-di-*cis*, 7,9,13-tri-*cis*.

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(nonpolar solvents) for more favorable formation of the 13-*trans* geometry (*1*) gave a mixture of isomers of the retinonitrile. The combined yield of **6** from **1** was 32%. A fraction containing only the 11-*cis* and 9,11-di-*cis* isomers of **6** was obtained after column chromatography over silica gel. Reaction of the mixture with diisobutylaluminum hydride gave the two corresponding retinal isomers. The 9,11-di-*cis* isomer was isolated by preparative hplc (25 cm, 5-μm silica gel columns).

The pmr spectrum (Fig. 1) of the compound confirmed the expected polyene geometry. Some of the important features useful for configurational assignments are: the geminal coupling constants, $J_{7,8}$ = 16.0 Hz and $J_{11,12}$ = 11.5 Hz (*trans* and *cis*, respectively) and the characteristic low-field absorptions of CH_3-5 (7-*trans*) (*2*), H-8 (9-*cis*) (*3*), and CH_3-13 (13-*trans*) (*4*, *5*) and high-field absorption of H-12 (11-*cis*) (*4*, *5*). The uv–vis absorption spectra of the new isomer and three other related isomers are shown in Fig. 2. The main band is expectedly blue shifted with respect to all-*trans*-retinal, but quite similar to that of 11-*cis*-retinal (*6*). The *cis*-band is not as prominent as in 11-*cis* but perhaps not inconsistent with the doubly bent polyene geometry.

Unlike isomers containing the 11,13-di-*cis* geometry, which provides a low-energy pathway for isomerization to the 11-*trans*, 13-*cis* geometry (*1*, *6*, *7*), 9,11-

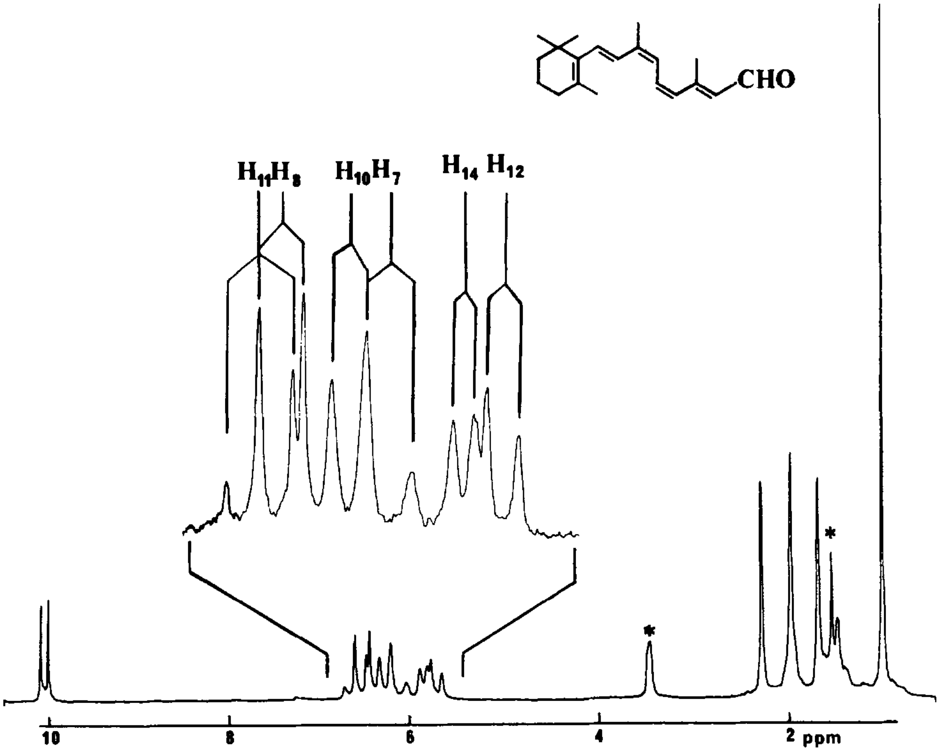

FIG. 1. pmr spectrum (Varian XL-100) of 9,11-di-*cis* retinal in CCl_4 with 5% dioxane-d_8. The peaks marked with an * are due to solvent impurities. The inset is the expanded vinyl region recorded at SW = 250 Hz.

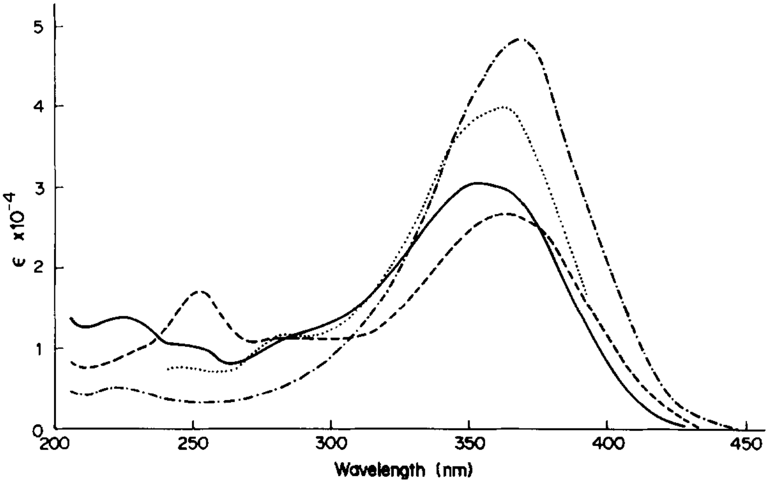

FIG. 2. uv–vis absorption spectrum of 9,11-di-*cis*-retinal (——) in hexane along with those of all-*trans*- (–·–), 9-*cis*- (·····), and 11-*cis*-retinal (– – –) (Hubbard, R., *J. Amer. Chem. Soc.*, 1956, **78**, 4662–4667). The λ_{max} and ϵ values for the four isomers are: 9,11-di-*cis*, 352 nm (30,600); all-*trans*, 368 nm (48,000); 9-*cis*, 363 nm (39,600); 11-*cis*, 363 nm (26,400).

di-*cis*-retinal appears to be thermally stable at room or lower temperatures. Two factors probably contributed to its stability. First, the activation energy for 6*e* electrocyclization of *cis*-1,3,5-hexatriene is higher than that of a *cis*-dienal. (*8*). Second, the 9,11-di-*cis* geometry in the 8-s-*cis*, 10-s-*cis* or 10-s-*cis*, 12-s-*cis* conformation (either necessary for 6*e* electrocyclization) appears to be more crowded than the corresponding conformers with the 11,13-di-*cis* geometry.

When incubated with cattle opsin solubilized with digitonin in a manner reported previously (*9*), 9,11-di-*cis*-retinal was found to give a pigment analog (Fig. 3). The rate of pigment regeneration is noticeably slower than with 11-*cis*-retinal, but comparable to those with the 7-*cis* geometry (*10*). The pigment yield was low: even with a twofold excess of the retinal, the yield was only ca. 20%. Similar to the rhodopsin analogs containing the 7-*cis* geometry, we found the new pigment only moderately stable in an excess of hydroxylamine (100 m*M*, at 25°C, the half-life of the pigment was 70 min). The difference spectrum (Fig. 4) of the rhodopsin analog, obtained by taking the difference between the pigment absorption spectrum recorded 50 min after the addition of hydroxylamine and that after bleaching with light ≥520 nm, clearly shows that it has an absorption maximum characteristically different from those of other related pigment isomers.

On the basis of the observations of slow rate of pigment formation, low yield, and relatively low stability in hydroxylamine, we conclude that the shape of the binding site associated with the most stable conformation of opsin must be significantly different from the shape of the new retinal isomer. Only after conformational readjustment the protein becomes compatible with the doubly bent retinal but still not to an extent to be able to shield the Schiff base linkage completely from attack by hydroxylamine.

Although we have not carried out experiments to establish rigorously that the

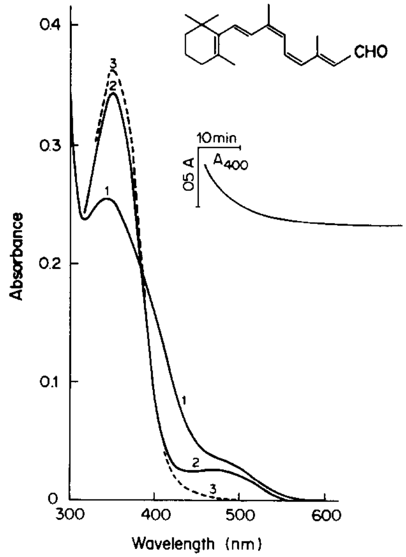

FIG. 3. A reaction mixture containing 0.45 *A* units of 9,11-di-*cis*-retinal and 0.22 *A* of cattle opsin in 1 ml of 1% digitonin buffer (pH 7.0) was incubated at 25°C for 12 hr. Then the mixture was diluted to 2 ml. The spectrum was measured (curve 1). Immediately after the addition of 50 m*M* hydroxylamine (in final concn), A_{400} was monitored (the insert). The slow decay after the first 10 min was attributed to instability of pigment in NH_2OH. Curve 2 was measured after 50 min. Subsequent irradiation (>520 nm) for 5 min (50-W tungsten lamp, glass cutoff filter) gave the curve 3.

9,11-di-*cis* geometry of the retinal remains intact in the pigment analog, the following observations suggest this is indeed the case. First, the absorption maximum of the pigment is different from that of any known rhodopsin isomers. Second, for the two rhodopsin isomers (9,13-di-*cis* (*11*) and 7,11-di-*cis* (*12*) where

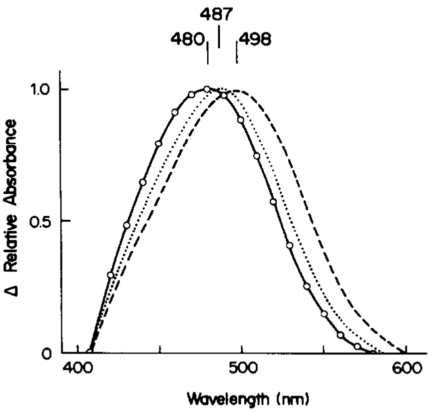

FIG. 4. The difference absorption spectra of 9,11-di-*cis*-rhodopsin (——), 9-*cis*-rhodopsin (····) (Hubbard, R., and Wald, G., *J. Gen. Physiol.*, 1952/1953, **36**, 269–315) and 11-*cis*-rhodopsin (– – –) (Kini, A., Matsumoto, H., and Liu, R. S. H., *J. Amer. Chem. Soc.*, 1979, **101**, 5078–5079). The absorption intensities were arbitrarily normalized identically at the λ_{max}.

the retinal was recovered by denaturing samples of the pigment analogs, the original geometry was found intact.

The end product of the photobleaching process is all-*trans*-retinal as indicated by the absorption maximum of the oxime. Presently, we have no information on the nature of the primary photochemical process, which is being studied at low temperatures in the laboratory of Professor Yoshizawa.

ACKNOWLEDGMENT

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