halogen in the species,[17] and a formulation $K_{1.75}Pt(CN)_4 \cdot X_{0.038} \cdot 1.5H_2O$ where X is Br^- or Cl^- is a possibility. We have searched for evidence of extra electron density in our Fourier maps and have attempted to refine very weak peaks (probably noise) with no success. The present stage of our crystallographic analysis suggests a halogen free complex with all platinum atoms being in the equivalent oxidation state of 2.25. The crystallographic properties of this system suggest the observation of high conductivity and possibly a metallic state at room temperature. The optical, electrical, and magnetic properties of this system are currently being investigated.

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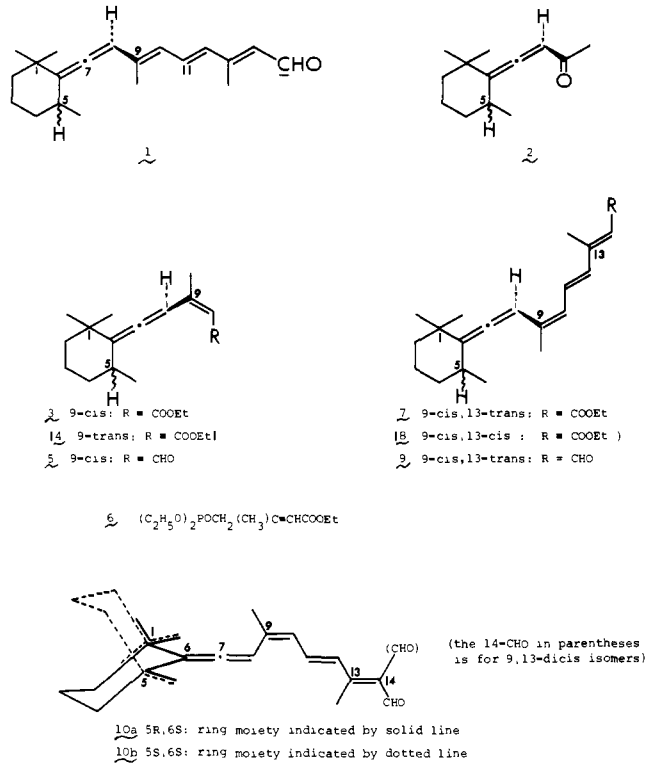
Allenic Retinals and Visual Pigment Analogues

Sir:

As part of our studies directed towards clarifying the steric factors involved in rhodopsin formation,[1,2] we have studied the spectral properties of artificial rhodopsin analogues prepared by the binding of bovine opsin to the various double bond isomers of 6,7-didehydro-5,6-dihydroretinals ("6-allenic retinals"), e.g., all-trans allenic retinal **1**.[3] These unique chromophores are of particular interest in studying the steric (including chiroptical) limitations imposed on the retinal moiety by the apoprotein opsin since: (i) the allenic bond fixes the cyclohexane ring at right angles with the pendant polyene thus providing models for comparing the role of twist about the 6-s-bond in normal retinals, and (ii) they contain two chiral centers, C-5 and C-6,[3] and therefore



enable one to gain information on the opsin chiroptical requirements.

The various double bond isomers were prepared separately by routes analogous to that exemplified in the following,[4] or by photoirradiation of the all-trans isomer (analogous synthesis) and separation by high-pressure liquid chromatography (HPLC).

Condensation of the known diastereomeric allenic ketone **2**[3,5] (with 2 mol of triethyl phosphonoacetate, *n*-butyllithium, ether, 25°, 3 days), and chromatography (silica gel, 30–50% CH_2Cl_2–hexane) of the product afforded diastereomeric 9-cis ester **3** (31%), 8-H at 7.52 ppm ($CDCl_3$), and the 9-trans ester **4** (53%), 8-H at 5.96 ppm. Reduction of the 9-cis ester **3** with diisobutylaluminum hydride (dibal) (hexane, −78°) followed by MnO_2 oxidation (hexane, 0°, 2 h) yielded the crude aldehyde **5**. Reaction of aldehyde **5** with phosphonosenecioate **6**[6] (NaH, THF, 0–25°) gave a mixture of the 13-trans/cis esters **7**[3] and **8**[3] (54% from **3**), which were separated by silica gel chromatography (30% CH_2Cl_2–hexane). Reduction of the 9-cis-13-trans ester **7** with dibal (hexane, −78°) and subsequent MnO_2 oxidation (CH_2Cl_2, 25°, 2 h) gave the crude diastereomeric 9-cis-13-trans aldehydes **9**,[3] 80% yield from ester **7**. HPLC,[1] μ-porasil, 1 ft × 0.25 in. (two), 1.5% ether in hexane, 2 ml/min flow rate, monitored at 350 nm, was employed to purify the respective retinals thus synthesized or to separate the double bond mixture resulting from photoisomerization of the all-trans isomer.

Both diastereomers of all-trans, 9-cis, 13-cis, and 9,13-dicis isomers could, respectively, be separated by running a second HPLC in 1% ether–hexane and at a flow rate of 1:1 ml/min (a few other peaks were present, presumably due to other isomers, but due to their minute quantity they were not pursued further). The double bond configurations of various allenic retinals thus obtained were determined by 270-MHz FT-NMR (Table I) by reference to the data for normal retinals.[7] All allenic retinals had uv spectra, in 3-methylpentane, consisting of a fine-structured triplet centered around 350 nm.[8]

Except for the 9-cis isomer, the three other isomers were

Table I. Chemical Shifts of Allenic Retinals, 270 MHz, $CDCl_3$, ppm from TMS

Allenic retinals[a]	1a-Me	1e-Me	5-Me	8-H	9-Me	10-H	11-H	12-H	13-Me	14-H	15-H
All-trans-I	1.18	1.08	1.00	6.03	1.93	6.17	7.10	6.36	2.37	6.00	10.11
All-trans-II	1.22	1.08	1.00	6.09	1.95	6.17	7.10	6.36	2.37	6.00	10.11
9-Cis-I	1.14	1.08	1.00	6.53	1.90	6.03	7.19	6.29	2.33	5.99	10.11
9-Cis-II	1.17	1.08	1.00	6.55	1.92	6.03	7.19	6.29	2.33	5.99	10.11
13-Cis-I	1.19	1.08	1.00	6.01	1.98	6.15	7.17	7.44	2.19	5.81	10.16
13-Cis-II	1.22	1.08	1.00	6.05	1.98	6.15	7.17	7.44	2.19	5.81	10.16
9,13-Dicis-I	1.19	1.08	1.00	6.49	1.98	6.15	7.28	7.38	2.19	5.81	10.16
9,13-Dicis-II	1.22	1.08	1.00	6.52	2.00	6.15	7.28	7.38	2.19	5.81	10.16

[a] In each diastereomeric pair, the isomers which are eluted earlier and later in the HPLC are designated I and II, respectively. The diastereomeric pairs had different chemical shifts for 1-axial-Me, 8-H, and 9-Me (excepting 13-cis), the other ^1H NMR peaks being superimposable.

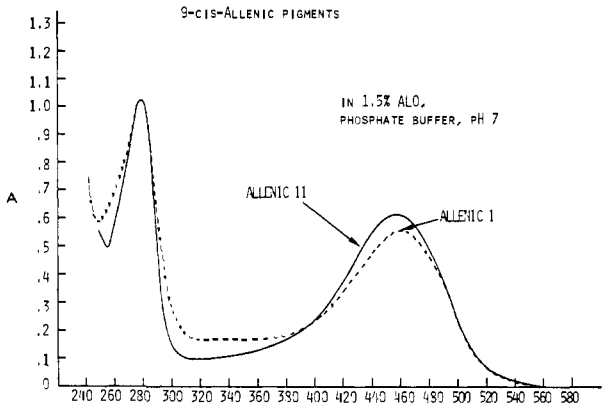


Figure 1. Absorption spectra of the two purified 9-cis allenic pigments, room temperature. Wavelength (nm) is plotted against absorption (normalized to 1.0 at λ_{max} of 280 nm).

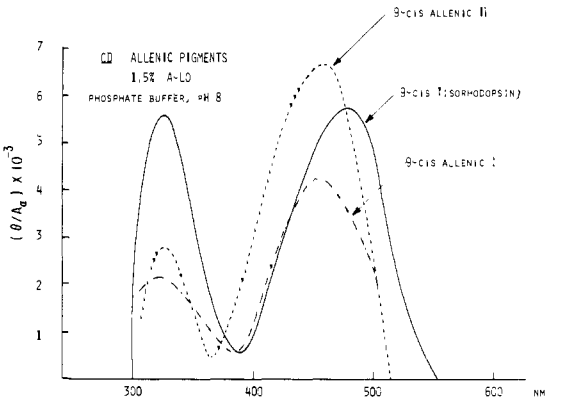


Figure 2. Circular dichroism spectra of the two purified 9-cis allenic pigments, room temperature. The CD of 9-cis rhodopsin[2] is also shown for comparison. Wavelength (in nm) is plotted against $(\theta/A_\alpha) \times 10^{-3}$, or ellipticity (in millidegrees)/absorption of α-bond: allenic I, 320 (θ/A 2.2) and 455 nm (θ/A 4.1); allenic II, 320 (θ/A 2.8) and 455 nm (θ/A 6.4).

used as the diastereomeric mixture in the following experiments because of the limited amount available. Each isomer was incubated with opsin[9,10] and pigment formation was checked by monitoring for a new absorption band above 400 nm. Of the four isomers available, only the 9-cis and the 9,13-dicis isomers were found to bind with opsin to form pigments.[11]

The respective pigments formed from 9-cis-allenic I (early HPLC peak), 9-cis-allenic II (late HPLC peak), unseparated ca. 1:1 diastereomeric mixture of 9-cis-allenic I and II, and unseparated ca. 1:1 diastereomeric mixture of 9,13-dicis allenic retinals were purified by calcium phosphate chromatography, 1.5% Ammonyx LO.[12] A portion of the pigments was then submitted to the mild denaturation-extraction procedure with methylene chloride[13] and HPLC in order to ascertain that the chromophores had not isomerized during pigment formation. The absorption and CD spectra of the pigments formed from the two 9-cis isomers are shown in Figures 1 and 2; the uv-visible spectra are almost identical, λ_{max} 462 nm for allenic I and λ_{max} 456 nm for allenic II pigment. The 9,13-dicis pigment derived from the mixed diastereomers had λ_{max} 455 nm, and CD 325 nm (θ_{325}/A_{455} 1.8) and 455 nm (θ_{455}/A_{455} 5.0). The pigments formed from the diastereomeric mixture of both 9-cis allenic retinal and 9,13-dicis allenic retinal gave the same ca. 1:1 peak ratio of diastereomeric retinals after detachment from the protein. Thus opsin appears to bind equally well with both members of the diastereomers, a surprising finding.

The three pigments derived from 9-cis-I, 9-cis-II and diastereomeric 9,13-dicis allenic retinals were then bleached by exposure to light. The α and β bands had now disappeared but a very small amount of optical activity was left at ca. 350 nm, a fact which could be attributed to a slight preferential binding for one of the diastereomers. The HPLC fraction of the chromophores extracted from the pigment with CH_2Cl_2 had little or no CD extrema.[14] We conclude therefore that *the opsin had bound with both antipodes to almost the same extent.*[14]

The two diastereomers, 5*R*,6*S* and 5*S*,6*S*, are collectively depicted in structure **10** for the 9-cis (and 9,13-dicis) allenic retinals. Structures for the antipodes can be visualized by moving the C-1 axial methyls (solid and dotted lines) to C-5. In view of the unexpected finding that the protein does not differentiate between the two diastereomers **10a** and **10b,** it is not surprising that it binds almost equally with both antipodes, since drawing **10** shows that the difference arising from the C-1 vs. C-5 axial methyls (antipodes) is already incorporated in the solid and dotted framework of the two substituted cyclohexane rings. The criteria for rhodopsin formation with respect to the ring moiety of retinal thus appears to be much more lenient than generally believed. Recent findings[1,2,11] have shown that opsin will bind with the bent chromophores, 7-cis, 9-cis, 11-cis, and 9,13-dicis (but not with extended all-trans and 13-cis, including the allenic retinals described here); it is also known that some other modified retinals afford rhodopsin analogues.[15] Studies are in progress to obtain a clearer picture of the steric requirements for pigment formation.[16]

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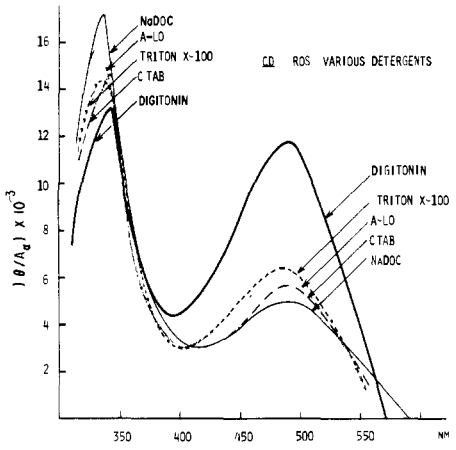

Figure 1. Circular dichroism spectra of bovine rod outer segments (ROS) in various detergents, room temperature. Wavelength is plotted against $(\theta/A_\alpha) \times 10^{-3}$, or ellipticity in millidegrees of α (or β) band/absorption of α band.

Table I. Absorption and Circular Dichroism Spectra

Detergent[a]	λ_{max}	Cotton effects[b] β	α	θ_{max}/A_α[c,d] β	α	R_α/R_β[e]
Digitonin[f]	498	340	490	12.6	11.7	0.87
Triton X-100	498	335	485	14.4	6.8	0.44
A-LO	498	330	490	16.1	6.3	0.42
CTAB	497	340	490	14.1	5.7	0.46
NaDOC	497	335	485	17.0	4.9	0.36

[a] Triton X-100, poly(oxyethylene (9∿10)octylphenol); A-LO, 2:1 mixture of lauryldimethylamine *N*-oxide (LDAO) and tetradecyldimethylamine *N*-oxide; CTAB, cetyltrimethylammonium bromide; NaDOC, sodium desoxycholate. All detergent concentrations were 50 mM, 67 mM phosphate buffer, pH 8.0. [b] Error ±3 nm. [c] Average of several runs. [d] θ, ellipticity in millidegrees of α (or β) CD band maximum; A, absorption of α band maximum. [e] R = rotational strength. [f] 2% w/v.

Micellar Effects on the Photochemistry of Rhodopsin

Sir:

The visual pigment rhodopsin is composed of an 11-cis retinyl chromophore[1,2] bound covalently via a protonated Schiff base linkage[3] to the ϵ-amino group of a lysine[4-6] in the apoprotein ospin. Rhodopsin has absorption band maxima at 498 (ϵ 40 600), 350, and 280 nm, and positive circular dichroism bands at 490 (α-band), 340 (β-band), and 270 nm.[7-9] Upon absorption of a photon of light by rhodopsin, the 11-cis chromophore is isomerized to the all-trans form with a quantum efficiency of 0.67.[10] At room temperature, all-trans retinal and opsin are the reported products of this bleaching process; however, a series of thermal intermediates has been characterized at lower temperatures.[11]

During our studies on visual pigments[12,13] it was observed that cis retinals could be isolated from the bleaching of rhodopsin and that the amount of cis retinal formed varied with detergents as well as with the irradiation time. Since most of the spectral and photochemical studies of rhodopsin have been carried out in various detergents, it appeared important to study the spectral and photochemical properties of rhodopsin in several detergents in order to determine the extent that these properties are altered. Thus we have examined the circular dichroism spectra and photochemistry (resulting from pulsed laser excitation) of rhodopsin in several detergents.

Rod outer segments were obtained from bovine retinas (Hormel-Austin, Minn.) by the sucrose flotation method.[14] The rhodopsin solutions were in 67 mM phosphate buffer, pH 8.0 with detergent concentrations of 50 mM, with the exception of digitonin which was 2% (w/v). All solutions were prepared at 0°C under a dim red light.

The electronic absorption spectra (Table I), Cary 17, of the extracted rod outer segments were identical in all detergents. The circular dichroism (CD) spectra, JASCO J-40, scan rate 50 nm/min, optical density of 0.5–1.0 at 500 nm, 1-cm path length, were recorded twice in succession to verify that bleaching had not occurred. Although positions of the α- and β-bands are quite close in the five detergents (Table I, Figure 1) large variations were present in the values of θ_{max}/A_α (θ is molar ellipticity, A is optical density) and R_α/R_β (R is rotational strength) calculated from these spectra. The CD results are interesting since, regardless of whether the origin of rhodopsin optical activity is due to a twisted chromophore[7,8] and/or a dipole-dipole interaction,[15,16] they provide a sensitive measure of the secondary chromophore-protein interactions. The fact that the θ_{max}/A_α and R_α/R_β values vary with detergents shows that the micelle formed from these detergents affect the shape of rhodopsin, thus giving rise to different steric and/or electronic interactions with the chromophore.

Bleaching was carried out using one 460-nm excitation pulse from a tunable dye laser, Phase-R DL 2100 B, $t_{1/2} \sim$ 300 ns. The opsin was denatured and the chromophore was extracted[17] by addition of an equal volume of cold methylene chloride to the laser flashed rhodopsin solution through a no. 14 gauge syringe and emulsification by repeated suction-protrusion in the dark. The emulsion was centrifuged and the methylene chloride layer was separated, dried over sodium sulfate, and concentrated to 20–30 μl,