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Visual Pigment Analogs from Isomers of 5,6,7,8-Tetrahydroretinal. The Importance of the Trimethylcyclohexyl Ring

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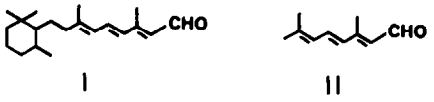
Three of the five isomers of 5,6,7,8-tetrahydroretinal were found to form pigment analogs when incubated with cattle opsin. Failure of dehydrocitral to form a similar pigment indicates the importance of the six-membered ring in pigment formation.

In our studies of the photochemistry of the polyenes in the retinal series (*1*, *2*), we prepared the nitrile and ester analogs of compounds **I** and **II** (Scheme 1). We have now prepared and isolated isomers of these compounds and studied their interaction with cattle opsin. The results, discussed below, are relevant especially in view of the current interest in the binding site requirements of opsin.

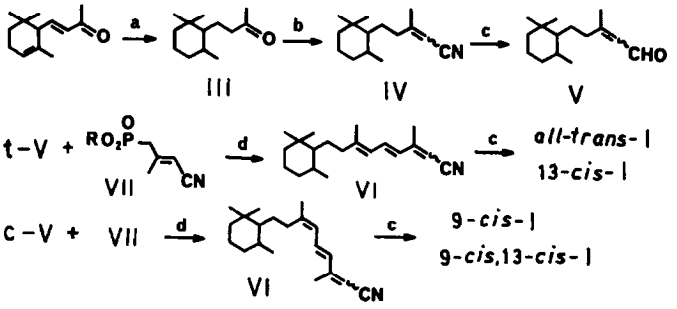


I II

SCHEME 1

RESULTS

5,6,7,8-Tetrahydroretinal (**I**) was prepared by catalytic hydrogenation of α-ionone to tetrahydroionone followed by chain extension reactions similar to those used in synthesis of vitamin A isomers and analogs (*3*) (Scheme 2). The 3:2



SCHEME 2. (a) H_2/Pd, (b) $(EtO)_2POCH_2CN$ + NaH, (c) DIBAL-H, (d) NaH.

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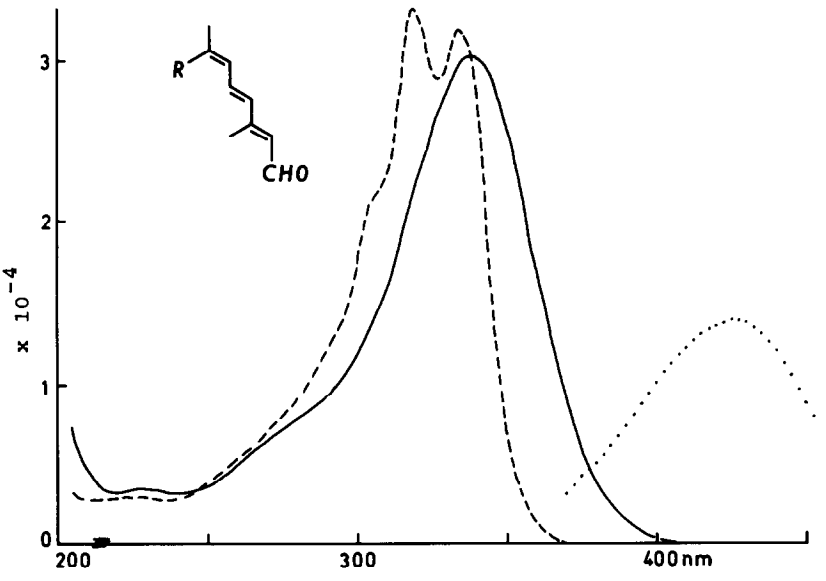


FIG. 1. The uv–vis absorption spectra of 9-*cis*-5,6,7,8-tetrahydroretinal and its pigment analog. In hexane (---), λ_{max} 319 nm, ϵ 3.30 × 10^4; in ethanol (—), λ_{max} 3.38 nm, ϵ 3.02 × 10^4. For the pigment analog (···), λ_{max} 426 nm, ϵ not determined.

trans–cis isomeric mixture of the C_{15} aldehyde **V** was separated by column chromatography. Condensation of either isomer with C_5 phosphonate **VII** gave two isomer mixtures of the C_{20} nitrile (**VI**). After partial reduction by diisobutyla-

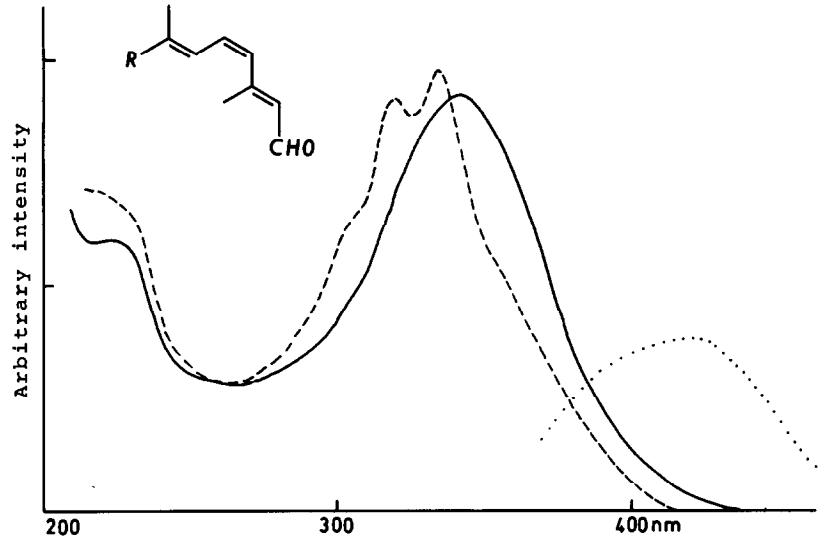


FIG. 2. The absorption spectrum of 11-*cis*-5,6,7,8-tetrahydroretinal and its pigment analog. In hexane (---), λ_{max} 333 nm; in ethanol (—), λ_{max} 341 nm. For the pigment, λ_{max} 424 nm. Insufficient amount to determine ϵ accurately.

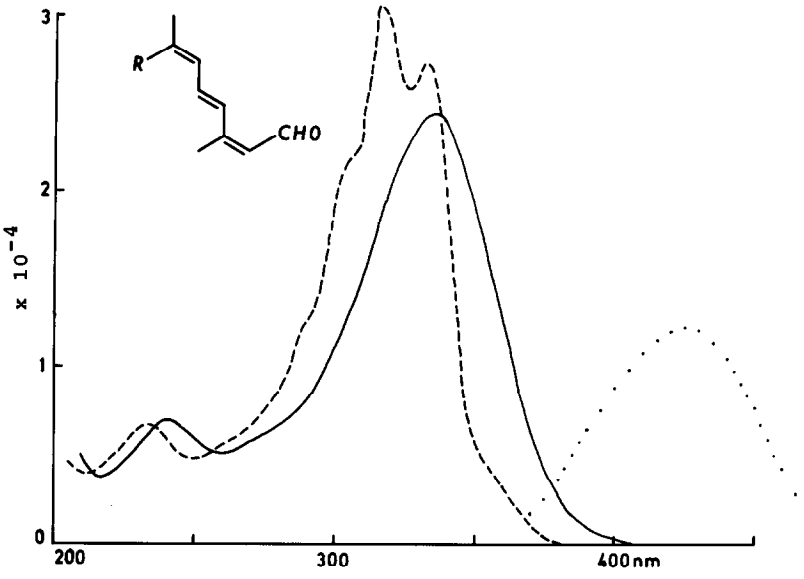


FIG. 3. The absorption spectra of 9-*cis*,13-*cis*-5,6,7,8-tetrahydroretinal and its pigment analog. In hexane (---), λ_{max} 316 nm, ϵ 3.05 × 10^4; in ethanol (—), λ_{max} 335 nm, ϵ 2.43 × 10^4. For the pigment, λ_{max} 426 nm, ϵ not determined.

luminum hydride (DIBAL-H), the C_{20} aldehyde isomers were isolated by high-pressure liquid chromatography.[1] By ^{1}H nmr, they were identified as the 13-cis, 9,13-dicis, 9-cis, and the all-trans isomers (see Experimental). Photoisomerization under a variety of conditions did not lead to isolable amounts of the 11-cis isomer. The latter was obtained by partial reduction of the 11-cis isomer of the corresponding nitrile, isolated from an irradiated mixture (*2*). The all-trans isomer of dehydrocitral was prepared according to the literature procedure (*4*). The 2-cis isomer was also isolated from the synthetic mixture. The 4-cis isomer was not detected in the synthetic nor in the irradiated mixtures.

The uv absorption spectra of three of the five isomers of **I** are shown in Figs. 1–3 with the data for the remaining two listed under Experimental. The ^{1}H-nmr data are also listed under Experimental.

Of the five isomers of **I**, the all-trans and the 13-cis isomers were found not to give pigment analogs. The 11-cis, the 9-cis, or the 9,13-dicis isomer when incubated with cattle opsin in the usual manner (*5*) was found to give a low yield of the pigment analog (6.5, 15, and 12%, respectively). The progress of the formation of the 9,13-dicis pigment is shown in Fig. 4. Curve 1 was recorded immediately after mixing of bovine rod outer segment (ROS) extract with an excess of the retinal analog isomer. Upon addition of a large excess of NH_2OH to a final concentration of 50 m*M*, the absorbance decrease at 360 nm was monitored (center). The initial

[1] In principle, each isomer may exist in diastereomeric forms. However, we have not been able to detect diastereomers under our HPLC conditions nor in their 200-MHz ^{1}H-nmr spectra (recorded at Nicolet Instrument).

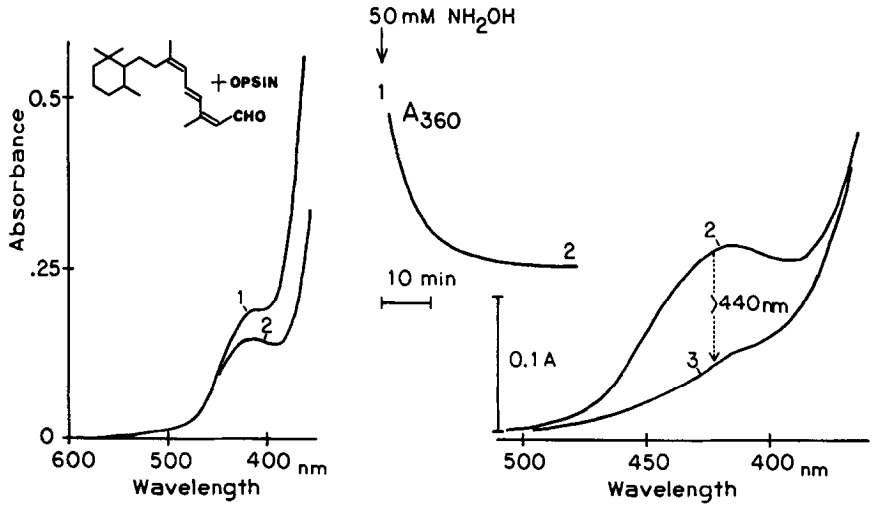


FIG. 4. Preparation of 9-*cis*,13-*cis*-5,6,7,8-tetrahydrorhodopsin. Left: Curve 1, absorption spectrum of a mixture of cattle opsin (sufficient to give 0.93 A unit of rhodopsin) solubilized in digitonin with 1.5 A unit of 9-*cis*,13-*cis*-I in EtOH; curve 2; same mixture 40 min after addition of an excess of NH_4OH. Middle: Absorbance decrease at 360 nm after addition of NH_2OH. Right: Curve 2; recorded on an expanded scale; curve 3, after irradiation with yellow light (450 nm). The difference spectrum for curves 2 and 3 is shown in Fig. 3.

rapid decrease of absorbance which is probably associated with removal of the random Schiff bases, ceased after ~20 min. The absorption spectrum (curve 2) was recorded after 40 min of addition of NH_2OH. Upon bleaching with yellow light (≥450 nm), curve 3 (right) was obtained. The difference of these two curves gave the absorption spectrum of the pigment analog. For the 9-cis and the 9,13-dicis isomers, the absorption maxima of the corresponding pigments centered at 426 nm, and that for the 11-cis isomer at 424 nm (see insert in Figs. 1–3).

Under the same conditions *trans,trans*-dehydrocitral, **II**, was found not to give detectable amounts of pigments.

DISCUSSION

In a study of the effect of the position of external point charges on absorption properties of visual pigments, Nakanishi and co-workers prepared a series of 9-*cis*-dihydroretinals (*6*). It is not surprising that the absorption properties of the tetrahydroretinals and rhodopsins are quite similar to that from 9-*cis*-7,8-dehydroretinal (420 nm). It is somewhat surprising, however, that in spite of the structural similarity between *trans,trans*-**II** and the 9-*cis*-**I** (and that of 7,8-dihydroretinal) the former did not give a pigment analog. This result is reminiscent of Blatz' work on 9,13-didemethylretinal, two of the cis isomers of which gave pigments while the related 2,4,6,8-decatetraenal did not (*7*). These results clearly show the importance of the trimethylcyclohexyl and trimethylcyclohexenyl rings

in the interaction of retinal with opsin. A related observation is the reported inhibition of rhodopsin formation by β-ionone and β-ionol (*8*) and long-chain analogs, (*9*), which was interpreted in terms of complex formation in the form of hydrophobic interaction between the trimethylcyclohexenyl ring and the protein, thus prohibiting entry of retinal to the binding site. The absence of the six-membered ring apparently made the hydrophobic interaction of **II** with the binding site of opsin unfavorable.[2]

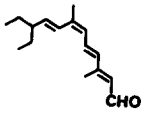
Superficially the stereoselectivity of opsin in interaction with isomers of **I** seems to parallel its interaction with the parent retinal isomers; namely, the all-trans and the 13-cis isomers do not form pigments, while 11-cis, 9-cis, and 9,13-dicis do (*11, 12*). There are clear differences, however, between these two systems. The presence of the 7,8-single bond in **I** should allow the compound to undergo rotation to a conformation resembling that of the 7-cis geometry. Since 7-*cis*-retinal and 7-*cis*,13-*cis*-retinal are known to form pigment analogs with cattle opsin (*13*), one might expect that all-trans and 13-cis isomers of **I** also would. The negative result is probably due to the cumulative negative effects of first modifying the polyene system to a tetrahydro system and then requiring it to convert to an unfavorable conformation for pigment formation. Secondly, it is interesting to note that the 11-cis isomer does not react with opsin any more easily than do the two 9-cis isomers. In fact, the yield of pigment analog is the lowest for 11-cis. We suspect that replacing double bonds with single bonds resulted in (plus other effects) lengthening of the overall molecular dimension, making 11-*cis*-**I** less compatible than retinal with the binding site of opsin (*14, 15*). It is interesting to note that in γ-retroretinals, opsin also exhibits a preference for the 9-cis isomer over the 11-cis (*16*).

EXPERIMENTAL

All nmr spectra were recorded on a Varian XL-100 spectrometer and uv–vis spectra on a Perkin-Elmer–Coleman 124 spectrometer. Procedures for isolation of ROS and formation of pigment analogs were identical to those described earlier (*3, 5*).

3,7-Dimethyl-2,4,6-octatrienal, **I.** The method of Lythgoe and Waterhouse (*4*) was used to synthesize 13.94 g of a heat- and oxygen-sensitive 2-cis/trans mixture

[2] A reviewer kindly brought to our attention the report by Crouch (*10*) of a visual pigment analog from the tetraenal shown. (Dr. Crouch has since informed us that the active isomer is the 9-cis



equivalent instead of the reported 11-cis.) While the compound does not have a cyclohexyl ring, it does contain a C_5 end group which occupies the identical positions as the carbons 1, 2, 4, and 5 of the six-membered ring. This report is therefore consistent with the current interpretation that a saturated end group is required in the hydrophobic interaction in pigment formation.

of the title compound. Bp 55–60° at 0.05 mm, lit.: 57–60° at 0.05 mm; uv (hexane) 331 nm.

5,6,7,8-Tetrahydroionone, **III.** A vessel with 90 g of crude α-ionone (Aldrich) and 1 g 10% Pd on C was fitted on a Parr hydrogenation apparatus, evacuated four times, and hydrogen introduced four times. After shaking for 2 days, the vessel was evacuated, air introduced, and the catalyst filtered off. The filtrate was distilled: bp 73°/0.4 mm yielding 83 g of the product. Its ^{1}H-nmr spectrum was consistent with the structure.

5,6,7,8-Tetrahydroionylideneacetonitrile, **IV.** To a 1-liter RBF[3] fitted with a septum inlet, a magnetic stirrer, nitrogen bubbler was added 7.68 g (1.6 eq) of oil-dispersed sodium hydride. The mixture was swirled and decanted four times with dry hexane. Fresh hexane (125 ml) was introduced, and the system was stirred and cooled to −23° under nitrogen. A dry dimethylformamide solution (75 ml) of 30 g (1.7 eq) of cyanomethylphosphonate was added dropwise by syringe. After completion of the addition, nitrogen bubbling was stopped and hydrogen evolution monitored while the flask reached room temperature. When the solid dissolved and gas generation appreciably slowed, the reaction was warmed to room temperature for 30 min. The flask was recooled under nitrogen to −23° and a hexane solution of 25 g of **III** was added dropwise and stirred overnight.

The mixture was poured into a separatory funnel containing about 100 ml of cool, dilute (3%, v/v) HCl. After the usual workup a colorless liquid was obtained. Upon vacuum distillation, 26.3 g (94%) (bp 93–105°, 50 μm) of **IV** was obtained. ^{1}H nmr ($CDCl_3$): the vinyl singlet at 5.05 ppm and allyl signals (1.85 and 2.00) were consistent with a 3/2 trans/cis mixture of the expected product.

5,6,7,8-Tetrahydroionylideneacetaldehyde, **V.** To a 1-liter RBF with a magnetic stirrer, a septum inlet, charged with 10 g of **IV** in hexane, cooled to −72° (CO_2(s), ethanol) under nitrogen, was added by syringe 55 ml (1.2 eq) of a 1 *M* hexane solution of DIBAL-H (Aldrich) and stirred. After 1 hr at room temperature, the solution was recooled to −72°, epsom salts ($MgSO_4 \cdot 7H_2O$) added to decompose excess hydride, gas evolution monitored, and stirred at room temperature until outgassing ceased. At −72°, 45 g wet silica gel was added in several portions, warmed to room temperature, and filtered. Evaporation of the solvent left 10.28 g of the crude product.

This residue was flash chromatographed (*17*) yielding 8.48 g (84%) of a colorless liquid. The ^{1}H nmr showed aldehyde doublets (J = 8 Hz) and vinyl doublets (J = 8 Hz), confirming structure **V**: ^{1}H nmr ($CDCl_3$) trans: 2.15 (3H), 5.80 (1H, J = 8 Hz), 10.02 (1H, J = 8 Hz); cis: 1.90 (3H), 5.72 (1H, J = 8 Hz); 9.89 (1H, J = 8).

The mixture was separated on a 150-cm × 12-mm column packed with 50 cm (1 liter) silica gel, and eluted with 4% ethyl acetate in hexane (v/v), taking 25 100-ml cuts, 88% recovery.

5,6,7,8-Tetrahydroretinonitrile, **VI.** The procedure for **IV** was followed with 1 g of either aldehyde from above, and a slight excess of the C_5 phosphonate **VII** (*2*), 1.14 g (89%) of 9-*trans*-tetrahydroretinonitrile mixture or 1.3 g (100%) of 9-*cis*-

[3] RBF, round bottom flask.

tetrahydroretinonitrile mixture was obtained. UV (hexane): λ_{max} 310 nm; ^{1}H nmr: containing expected signals for vinyl and allylic hydrogens for the products.

5,6,7,8-Tetrahydroretinals, **II** *(11-trans isomers).* These compounds were prepared by DIBAL-H reduction of the nitriles (see that for **V**). After flash chromatography, yields: 9-cis,13-cis 0.068 g; 9-cis 0.3416 g; trans plus 13-cis 0.0892 g. The isomers were purified by preparative HPLC (10-mm × 25-cm Lichrosorb column, 5% ether in hexane). UV: trans (hexane) ϵ (319 nm) 3.69×10^4 (334) 3.54×10^4, (EtOH) ϵ (338) 3.32×10^4; 13-cis: (hexane) λ_{max} 316, 332, (EtOH) λ_{max} 333 nm. For 9-cis, 11-cis, and 9,13-dicis isomers see Figs. 1–3. ^{1}H nmr (C_6D_6) all-trans: 1.70 (CH_3-9), 1.82 (CH_3-13), 6.0 (3H, overlapping multiplets), 6.81 (H-11, d × d, J = 11.5, 15.2 Hz); 10.06 (H-15, d, J = 8); 9-cis: 1.74 (CH_3-9), 1.86 (CH_3-13), 5.85 (H-10, d, J = 11.4), 6.02 (H-14, d, J = 8), 6.03 (H-12, d, J = 15.4), 6.89 (H-11, d × d, J = 11.4, 15.4), 10.08 (H-15, d, J = 8); 13-cis: 1.68 (CH_3-13), 1.70 (CH_3-9), 5.81 (H-14, d, J = 7.6), 6.02 (H-10, d, J = 10.8), 6.71 (H-11, d × d, J = 15, 10.8), 7.03 (H-12, d, J = 15), 10.18 (H-15, d, J = 7.6); 9,13-dicis: 1.75 (6H, CH_3-9, CH_3-13), 5.80 (H-14, d, J = 7), 5.86 (H-10, d, J = 10), 6.79 (H-11, d × d, J = 16, 10), 7.06 (H-12, d, J = 16), 10.20 (H-15, d, J = 7) (for ^{1}H nmr of retinal isomers see Ref. (*18*)).

11-cis-5,6,7,8-Tetrahydroretinonitrile, **VI.** An irradiated acetonitrile solution of the C_{20} nitrile was found to contain 20% of the 11-cis isomer (*2*). It was collected by preparative HPLC (½ in., 25-mm Lichrosorb column, hexane solvent). ^{1}H nmr ($CDCl_3$): 1.76 (CH_3-9), 2.24 (CH_3-13), 5.20 (H_{14}, br, s), 5.72 (H_{12}, d, J = 10), 6.25 (H_{10}, d, J = 10), 6.40 (H_{11}, br, t).

11-cis-5,6,7,8-Tetrahydroretinal, **II.** A 2-ml aliquot (2.32×10^{-5} mol) of a 5-ml hexane stock solution of the above isolated 11-*cis*-tetrahydroretinonitrile was reduced with DIBAL-H in the same manner as described above. The absence of uv active impurities (HPLC) allowed this sample to be used for opsin incubation work without purification. Its uv absorption spectra are shown in Fig. 2.

ACKNOWLEDGMENT

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