Opsin pigments formed with acyclic retinal analogues

Minimum 'ring portion' requirements for opsin pigment formation

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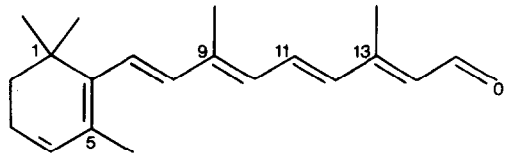
Three acyclic derivatives (3,7-dimethyl-10-ethyl-2,4,6,8-dodecatetraenal, 3,7,10-trimethyl-2,4,6,8-dodecatetraenal, and 3,7,10-trimethyl-2,4,6,8-undecatetraenal) have been synthesized and the 2-*cis*, 6-*cis*, 2,6-di*cis*, and all-*trans* isomers of each isolated. Unlike the undecatetraenal compound, the dodecatetraenal derivatives, have methyl groups which can mimic the C-1 and/or C-5 methyl group of retinal. The 6-*cis* and 2,6-di*cis* isomers of the dodecatetraenal compounds form photosensitive pigments with bovine opsin. No pigment is obtained with the undecatetraenal derivative. Therefore, although the retinal cyclohexyl ring is not essential for pigment formation, at least one methyl group corresponding to the C-1 or C-5 retinal methyl may be required for obtaining stable opsin pigments.

Rhodopsin *Visual pigment* *Retinal* *Isorhodopsin* *Regeneration* *Vitamin A*

1. INTRODUCTION

Rhodopsin, the photosensitive pigment of the vertebrate retina, is a lipoprotein linked to its chromophore, the 11-*cis* isomer of retinal **1**, via a protonated Schiff base. The steric and electronic constraints of the binding site of retinal have been investigated by several groups using retinal analogues [1]. The retinals found to form reasonably stable pigments with the apoprotein op-



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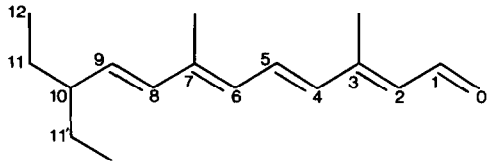

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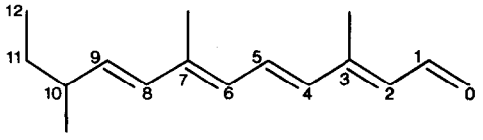

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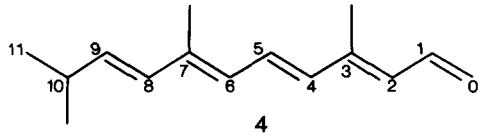

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sin all have two features in common:

(i) At least one bond in the side chain other than at C-13 which can assume a *cis* conformation;

(ii) At least one methyl group on the ring at C-1 or C-5.

Studies on the competitive inhibition of the rate of rhodopsin regeneration in the presence of compounds resembling portions of the retinal structure have also suggested that the ring methyls are of importance for stable interaction of the chromophore with the protein [2,3]. In [4] we reported that an acyclic derivative **2** lacking the cyclohexyl ring, but containing two methyl groups which can possibly mimic the C-1 and C-5 methyl groups of retinal, forms a stable photosensitive pigment with bovine opsin. Thus, the cyclohexyl ring of retinal is not essential for pigment formation but one or more of the ring methyl groups may be required for obtaining stable pigment. Here, we report the synthesis of two retinal analogues, one (**3**) containing a single methyl and the other (**4**) lacking any methyl which might fill the space requirements of the retinal ring methyls, and we describe studies on pigment formation between these analogues and bovine opsin.

2. EXPERIMENTAL

3,7-Dimethyl-10-ethyl-2,4,6,8-dodecatetraenal **2** is synthesized as in [4]. The intermediate, 6-ethyl-3-methyl-2,4-octadienal, is separated into the 2-*cis* and all-*trans* isomers by flash column chromatography [5] (10% ether/hexane). The 2-*cis* intermediate isomer forms the 6-*cis* and 2,6-di*cis* isomers of **2**; the all-*trans* intermediate forms the 2-*cis* and all-*trans* isomers of **2**. The 2-*cis*, 6-*cis*, 2,6-di*cis* and all-*trans* isomers, obtained in a ratio of 2:2:1:3 respectively, are separated by thin layer chromatography (silica gel, 5% ethyl acetate/hexane) and high-pressure liquid chromatography (HPLC, 1–5% ethyl ether/hexane, μ-Porasil column). The isomers are identified by nuclear magnetic resonance spectroscopy (NMR, table 1). 3,7,10-Trimethyl-2,4,6,8-dodecatetraenal **4** is syn-

Table 1

NMR data of acyclic retinal analogues

Analogue	Chemical shift δ (ppm)							
	3-CH_3 (*S*)	H_9 (dxd)	H_8 (d)	H_6 (d)	H_5 (dxd)	H_4 (d)	H_2 (d)	H_1 (d)
All-*trans* **2**	2.29	5.59	6.09	6.11	7.08	6.32	5.94	10.07
2-*cis* **2**	2.11	5.60	6.11	6.15	6.98	7.23	5.81	10.17
6-*cis* **2**	2.31	5.61	6.59	6.00	7.21	6.26	5.94	10.07
2,6-di*cis* **2**	2.13	5.62	6.58	6.04	7.10	7.18	5.82	10.17
All-*trans* **3**	2.30	5.73	6.10	6.11	7.08	6.32	5.94	10.07
2-*cis* **3**	2.11	5.73	6.12	6.15	6.98	7.23	5.81	10.17
6-*cis* **3**	2.31	5.73	6.61	6.01	7.21	6.26	5.94	10.08
2,6-di*cis* **3**	2.13	5.73	6.60	6.04	7.10	7.17	5.81	10.17
All-*trans* **4**	2.30	5.83	6.11	6.12	7.08	6.32	5.94	10.07
2-*cis* **4**	2.13	5.83	6.12	6.15	6.96	7.23	5.82	10.17
6-*cis* **4**	2.31	5.83	6.62	6.01	7.20	6.26	5.94	10.08
2,6-di*cis* **4**	2.13	5.83	6.60	6.05	7.09	7.19	5.85	10.17

Additional chemical shifts δ (ppm): **2**, **3** and **4**, 7-CH_3 = 1.95; **2**, 0.85 (11,11′-CH_3, *t*, J = 7.4), 1.38–1.21 (H_{11},*m*), 1.56–1.38 (H_{11}′,m), 1.95–1.85 (H_{10},m); **3** = 0.87 (11-CH_3, *t*, J = 7.4), 1.03 (10-CH_3, *d*, J = 6.7), 2.23–2.13 (H_{10},m); **4**, 1.00 (10-CH_3, *d*, J = 6.7); 2.53–2.36 (H_{10},*m*)

Coupling constants (Hz): **2**, **3** and **4**: $J_{8,9}$ = 15.5, $J_{5,6}$ = 11.3, $J_{4,5}$ = 15.0, $J_{1,2}$ = 8.1; $J_{9,10}$: **2** = 9.0, **3** = 8.0, **4** = 6.8

Recorded on a Bruker WH-400 Fourier transform NMR spectrometer at 400 MHz on solutions in $CDCl_3$ containing $CHCl_3$ (δ = 7.24 ppm) as internal standard. Chemical shifts are reported to a precision of ± 0.01 ppm and coupling constants to ± 0.2 Hz

thesized in a similar manner from 2-methylbutanal in an overall yield of 15%. The isomeric mixture shows a parent peak in the mass spectrum at *m/e* 218 (70 eV). The 2-*cis*, 6-*cis*, 2,6-di*cis* and all-*trans* isomers are separated as above and identified by NMR (table 1). 3,7,10-Trimethyl-2,4,6,8-undecatetraenal **4** is synthesized from 2-methyl propanal in an overall yield of 18%; mass spectrum M^+ *m/e* 204, 70 eV. The 2-*cis*, 6-*cis*, 2,6-di*cis* and all-*trans* isomers are separated as above and identified by NMR (table 1). Absorption data for all analogues is given in table 2; all isomers of the acyclic analogues showed fine structure in hexane which was not observed in ethanol.

Bovine rod outer segments are isolated from frozen retinae [6]. Pigment is regenerated from rods suspended in potassium phosphate buffer (67 mM, pH 7.4) by incubating with the retinal (10 molar excess) for 1 h at 25°C. Excess chromophore is removed by washing the pigment with 1% bovine serum albumin in potassium phosphate buffer. Pigment formation is measured by difference absorption spectra obtained by subtraction of the absorption spectrum after bleaching (exposure of the pigments to white light for 1 min) from the absorption spectrum obtained on pigments before bleaching. Stability of the pigment to hydroxylamine is assessed by the addition of 20 μl of a 1 M solution (pH 7.4) of NH_2OH to the pigment in suspension (~0.5 mg in 1 ml potassium phosphate (pH 7.4) and following changes in the absorption spectrum over 1 h. Stability of the pigment to 11-*cis* retinal is determined by the addition of a 5 M excess of the retinal to a pigment suspension and following changes in the absorption spectrum over 24 h.

Table 2

Absorption of acyclic retinal analogues and opsin pigments

Analogue	λ_{max} (nm)		Pigment λ_{max} (nm)
	Ethanol	Hexane	
All-*trans* **2**	366	347	–
2-*cis* **2**	359	344	–
6-*cis* **2**	360	343	460
2,6-di*cis* **2**	358	340	460
All-*trans* **3**	364	347	–
2-*cis* **3**	361	344	–
6-*cis* **3**	350	344	455
2,6-di*cis* **3**	358	346	453
All-*trans* **4**	364	346	–
2-*cis* **4**	358	343	–
6-*cis* **4**	358	341	–
2,6-di*cis* **4**	356	339	–

All analogues show fine structure in hexane (see fig.1 for 6-*cis* **3**) which is not detectable in ethanol. The λ_{max} of the pigments are obtained from difference absorption spectra measured by subtraction of the absorption spectra after exposure of the pigment to light for 1 min from spectra of the pigment before exposure (67 mM potassium phosphate buffer, pH 7.2). All values are ± 2 nm

3. RESULTS AND DISCUSSION

The 6-*cis* and 2,6-di*cis* isomers of **2** and **3** (corresponding to the 9-*cis* and 9,13-di*cis* isomers of retinal) form pigments with bovine opsin (fig.1). The λ_{max} of these pigments (table 2) are somewhat blue shifted from those of isorhodopsin (485 nm) [7] and isorhodopsin II (484 nm) [8] due to the lack of the C-5 'ring' double bond. Pigment is not obtained with any isomer of **4** even on prolonged incubation with bovine opsin. The acyclic pigments

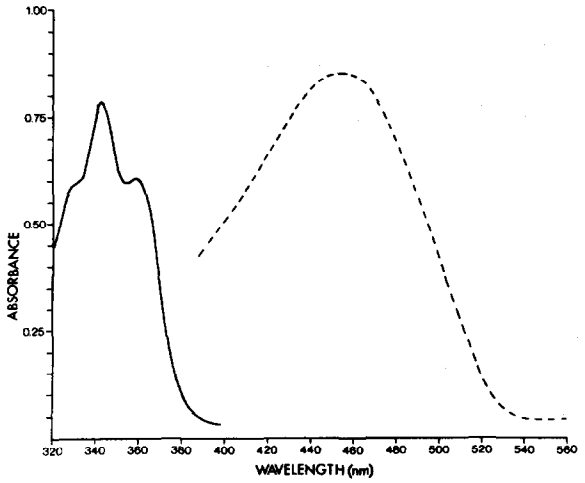

Fig.1. Absorption spectra of 6-*cis* 3,7,10-trimethyl-2-4,6,8-dodecatetraenal **3** in hexane (——) and pigment formed on combination of 6-*cis* **3** with an excess of bovine opsin in 67 mM potassium phosphate buffer (pH 7.4) (– – –).

are photosensitive to white light. Proof of the acyclic structure and the isomeric identity of the attached chromophore in the synthetic pigments was obtained by extraction of the chromophore in methylene chloride [8] and identification of the retinal by absorption and chromatographic data. The rate of pigment formation with both the acyclic 6-*cis* isomers of **2** and **3** is about 3-fold slower than with 9-*cis* retinal.

Addition of 11-*cis* retinal to the analogue pigments does not initially result in any decrease in the quantity of the analogue pigment or in the formation of rhodopsin. Therefore these acyclic retinal analogues appear to be binding at the native binding site for 11-*cis* retinal. However, over 24 h there is evidently some displacement of the 6-*cis* **3** by 11-*cis* retinal as the absorption at 500 nm increases with some concurrent decrease at 460 nm. The 6-*cis* **2** pigment spectrum is unaffected by 11-*cis* retinal over 24 h. The pigments formed from **2** were stable to the addition of hydroxylamine over 24 h. However, pigments formed from **3** decomposed over 5 h on the addition of hydroxylamine. These results suggest the second methyl group on the analogue adds to the stability of the pigment.

These data are evidence for the hypothesis that at least one ring methyl group of retinal is required for stable pigment formation. Within the opsin binding cavity, the retinal sites which provide the essential interaction between the protein and chromophore and thus determine pigment formation may be, in addition to the C-15 carbonyl, in the region of the C-1 and/or C-5 methyl groups.

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