Conformational Study of Retinochrome Chromophore: Synthesis of 8,18-Ethanoretinal and a New Retinochrome Analog†

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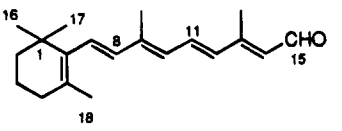
In order to investigate the chromophore conformation around the trimethylcyclohexene ring in retinochrome, (*all-E*)-8,18-ethanoretinal (**2**), in which C_8 and C_{18} positions of retinal **1** was connected by an ethylene group, was synthesized *via* the Dieckmann condensation of diester **6** from 2,2-dimethylcyclohexanone **7**. This analog **2** was incorporated into aporetinochrome, forming a pigment with λ_{max} at 498 nm. Its opsin shift (2200 cm^{-1}) is of the same order of magnitude as that of native retinochrome (2400 cm^{-1}), suggesting that the conformations of both chromophores are fairly similar in the protein. In addition, MMX calculations indicate that the torsional angle around the 6–7 single bond in retinochrome might correspond to the allowable torsional angle in 8,18-ethanoretinal (**2**).

The visual cells of cephalopods contains two kinds of photosensitive pigments, rhodopsin and retinochrome.[1] The most remarkable difference between these photopigments lies in the stereoisomeric form of their chromophore retinals, which is 11*Z* in rhodopsin but all-*E* in retinochrome. When they absorb light, their chromophores are changed to all-*E* and 11*Z* forms, respectively. Recently, it was shown that retinochrome plays an important role in supplying 11*Z* retinal in the regeneration of cephalopod rhodopsin.[2] In retinochrome, the chromophore **1** is bound to the ϵ-amino group of a lysine moiety of the apoprotein opsin through a protonated Schiff base (PSB).[3] The absorption maximum of retinochrome (496 nm) is much longer than that of the model PSB (443 nm) formed from all-*E* retinal and *n*-$BuNH_2$. The red shift in the absorption maximum of retinochrome relative to the model PSB is due to interaction of the chromophore with the protein chain, and the difference in wavenumbers (2400 cm^{-1}) has been called the "opsin shift".[4] One of the factors that contributes to the opsin shift is the conformation of the chromophore around the 6–7 single bond. Recently, we clarified that the chromophore in retinochrome has the 6s-*cis* conformation by use of 6s-*cis* and 6s-*trans* fixed retinal analogs.[5] Examination of Dreiding molecular models suggests that the torsional angle around the cyclohexene ring and the polyene side chain in 8,18-ethanoretinal (**2**), in which the C_8, C_{18} positions of **1** are connected by an ethylene group, is about 50–55°, and this value is seems to be very close to that in the crystal structure of **1**.



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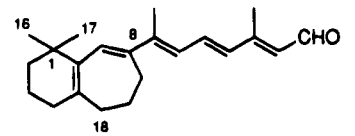

2

In order to estimate the above consideration, we describe here the full account of the preparation of 8,-18-ethanoretinal (**2**) mentioned in our previous paper[6] and its MMX calculation and also discuss the conformation of the chromophore in retinochrome.

Results and Discussion

The methodology for the transformation of a β-ionone analog to the corresponding retinal is already well established.[7] Thus, our retrosynthetic plan to 8,18-ethanoretinal (**2**) entails the use of β-ionone analog **3** as shown in Scheme 1. The key intermediate **3** would be available by either an aldol condensation of the formyl ketone **4** (route A) or introduction of a C2 unit to the bicyclic ketone **5**, which could be obtained by Dieckmann condensation of diester **6** and subsequent deethoxycar-

† Retinoids and Related Compounds. 17. For part 16, see: Katsuta, Y.; Ito, M.; Yoshihara, K.; Nakanishi, K.; Kikkawa, T.; Fujiwara, T. *J. Org. Chem.* **1994**, *59*, 6917.

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(1) Hara, T.; Hara, R. *In Handbook of Sensory Physiology*; Springer: Berlin, 1972; Vol. 1, Part 1: Photochemistry of Vison.

(2) Ozaki, K.; Terakita, A.; Hara, R.; Hara, T. *Vision Res.* **1987**, *27*, 1057. Hara, R.; Hara, T.; Ozaki, K.; Terakita, A.; Eguchi, G.; Kodama, R.; Takeuchi, T. *Retinal Proteins*; VNU Science Press: Utrecht, 1987. Terakita, A.; Hara, R.; Hara, T. *Vision Res.* **1989**, *29*, 639.

(3) Hara-Nishimura, I.; Matsumoto, T.; Mori, H.; Nishimura, M.; Hara, R.; Hara, T. *FEBS Lett.* **1990**, *271*, 106.

(4) Mott, M. G.; Sheves, M.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1980**, *102*, 7947.

(5) Kinumi, T.; Tsujimoto, K.; Ohashi, M.; Hara, R.; Hara, T.; Ozaki, K.; Sakai, M.; Katsuta, Y.; Wada, A.; Ito, M. *Photochem. Photobiol.* **1993**, *58*, 409.

(6) Wada, A.; Sakai, M.; Kinumi, T.; Tsujimoto, K.; Ito, M. *Tetrahedron Lett.* **1993**, *34*, 1069.

(7) Dawson, M. I.; Okamura, W. H. *Chemistry and Biology of Synthetic Retinoids*; CRC Press: Boca Raton, FL, 1990. Sporn, M. B.; Roberts, A. B.; Goodman, D. S. *The Retinoids*; Academic Press: London, 1984; Vol. 1.

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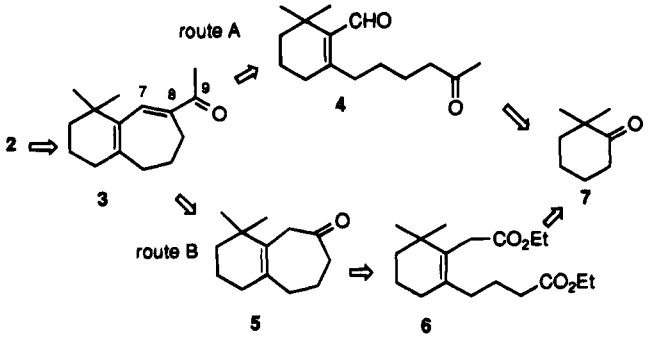
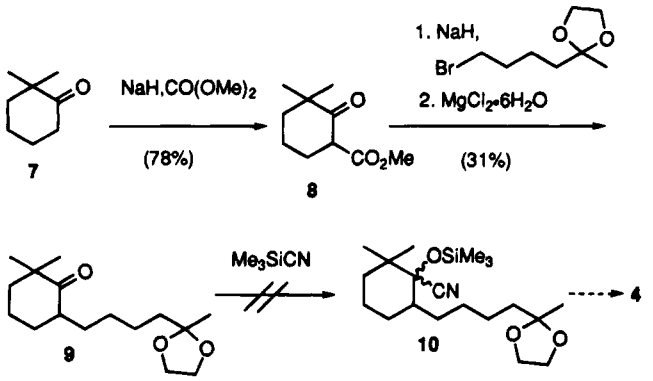
Scheme 1



Scheme 2



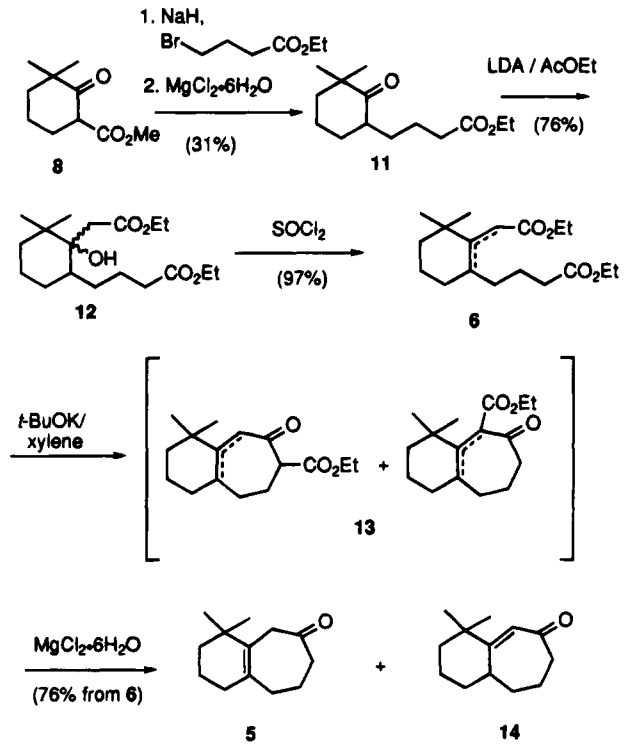
bonylation (route B). We employed 2,2-dimethylcyclohexanone (**7**) as starting material for both routes.

In the initial attempt using route A (Scheme 2), since attempts for the direct synthesis of **9** from the reaction of **7** with 1-bromo-5,5-(ethylenedioxy)hexane[8] were unsuccessful, **7** was converted to the β-keto ester **8** by the reaction with dimethyl carbonate (78%). Introduction of a 5,5-(ethylenedioxy)hexanyl chain into **8** occurred smoothly, and subsequent demethoxycarbonylation[9] gave the keto acetal **9** (31%). Treatment of **9** with trimethylsilyl cyanide in the presence of zinc dichloride as catalyst[10] afforded the complex mixture, of which the requisite adduct **10**, the precursor of **4**, was not detected at all. Accordingly, alternative route B (Schemes 3 and 4) was investigated.

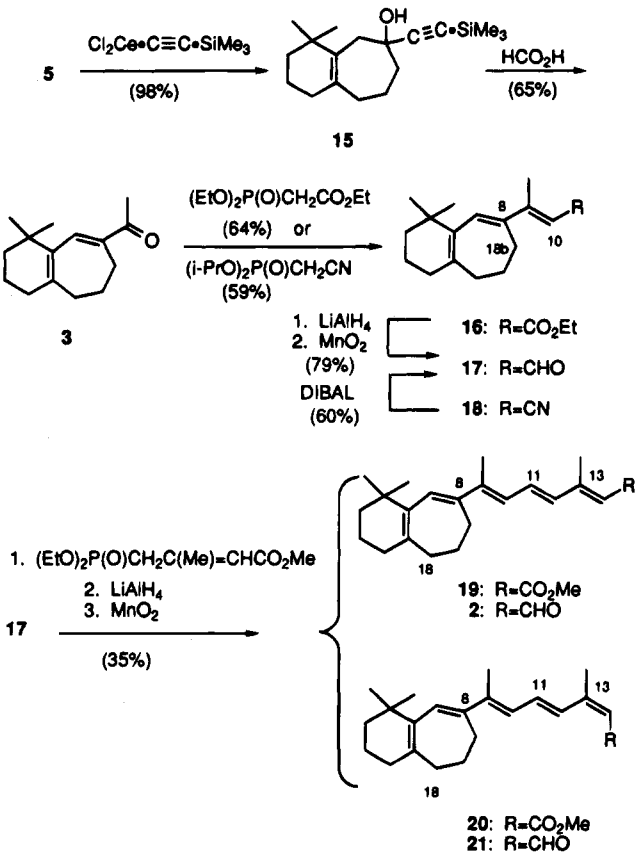
Introduction of an ethoxycarbonyl chain into **8** and subsequent demethoxycarbonylation was achieved in the same manner described above to provide the keto ester **11**. Treatment of **11** with ethyllithium acetate gave the adduct **12** in 76% yield as a mixture of stereoisomers which, without separation, was dehydrated with thionyl chloride to yield the diester **6** (97%). The ratio of **6** was determined by gas chromatographic analysis to be *endo*:*exo* = 2.4:1. The Dieckmann condensation[11] of **6** under the high dilution conditions in xylene using potassium *tert*-butoxide gave a mixture of cyclization products **13**, which without separation was deethoxycarbonylated to give the bicyclic ketone **5** (75%) accompanied by its regioisomer **14** (1%).

Our first attempt for the introduction of a C2 unit to the ketone **5** using lithium (trimethylsilyl)acetylene[12] was

Scheme 3



Scheme 4



unsuccessful, probably resulting from enolate formation. Since organocerium reagents are known to circumvent this problem,[13] **5** was treated with [(trimethylsilyl)-ethynyl]cerium(III) reagent, prepared from [(trimethyl-

(8) Anderson, E. P.; Crawford, J. V.; Sherrill, M. L. *J. Am. Chem. Soc.* **1946**, *68*, 1294.
(9) Tsuda, Y.; Sakai, Y. *Synthesis* **1981**, 119.
(10) Evans, D. A.; Truesdale, L. K.; Carroll, G. L. *J. Chem. Soc., Chem. Commun.* **1973**, 55. Evans, D. A.; Hoffman, J. M.; Truesdale, L. K. *J. Am. Chem. Soc.* **1973**, *95*, 5822.
(11) Schaefer, J. P.; Bloomfield, J. J. *Org. React.* **1967**, *15*, 1.
(12) Hanzawa, Y.; Suzuki, M.; Kobayashi, Y.; Taguchi, T. *Chem. Pharm. Bull.* **1991**, *39*, 1035.

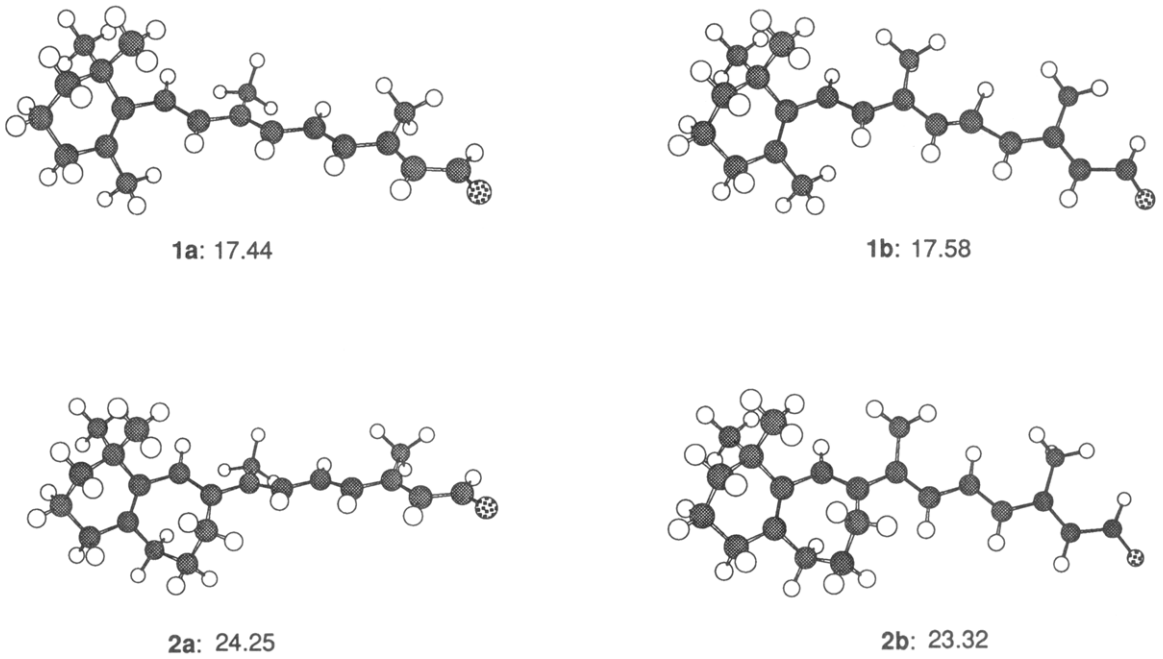


Figure 1. Different conformers of retinal (**1**) and 8,18-ethanoretinal (**2**) (steric energies in kcal/mol).

silyl)ethynyl]lithium and cerium(III) chloride in THF. The alcohol **15** was thus obtained in quantitative yield. Transformation of **15** to the β-ionone analog **3** was achieved according to the previously reported method[14] with formic acid.[15] Condensation of **3** with triethyl phosphonoacetate (NaH, THF) provided the ester **16**, which was converted to the aldehyde **17** by $LiAlH_4$ reduction and subsequent MnO_2 oxidation. Alternatively, reaction of **3** with diisopropyl (cyanomethyl)-phosphonate (NaH, THF) gave the nitrile **18**, followed by reduction with DIBAL to afford the aldehyde **17**. The *trans* geometry of newly produced double bonds of **16** and **18** was established from the strong NOESY correlation between 10-H and 18b-H in their NMR spectra.

The Emmons–Horner reaction of **17** with C5-phosphonate was carried out using *n*-BuLi to give the ester **19** and its 13*Z* isomer **20**. Determination of the stereochemistry (*E*-form) of the 11,12 double bond in **19** and **20** was based on the coupling constants of the 11-H signal in their NMR. It is noteworthy that although the C5-phosphonate was used as a mixture of double bond (*ca.* 1:1) in the condensation, the ratio of all-*E* isomer in the products increased dramatically (**19**:**20** = *ca.* 7:1). A similar result has been already reported by Gedye and co-workers.[16] Final transformation of **19** and **20** to the corresponding aldehydes **2** and **21** was established by $LiAlH_4$ reduction and MnO_2 oxidation according to the usual method. These isomers were isolated in pure form, respectively, by repeating the preparative HPLC in the dark.

For the evaluation of the structural conformation[17] of **2** and **1**, molecular mechanics calculations[18] were carried out. The conformation of the cyclohexene ring is fixed as shown in Figure 1. The conformers having a negative torsional angle between C_5-C_6 and C_7-C_8 planes are more stable (>2 kcal/mol) than those having a positive torsional angle. In the conjugated side chain, the torsional angle between C_7-C_8 and C_9-C_{10} planes slips out of the conjugated plane and has only a small effect on the steric energy (**1a** and **1b**). Although many conformations are possible in the case of ethanoretinal **2** due to the flexibility of bicyclo[5.4.0] system, the conformers other than those having the conformation of bicyclo[5.4.0]-nonadiene shown in Figure 1 can be assumed to have large steric energies significantly. Thus, the torsional angle of $C_7-C_8-C_9-C_{10}$ bends out of the conjugated plane more than those in **1** owing to the bulky ethylene bridge. Structural parameters of X-ray analysis of **1**[19] and molecular mechanics calculations of **1** and **2**, which have a similar conformation as X-ray, are summarized in Table 1. The good agreement between the calculated and X-ray crystal values of **1** is satisfactory in that most of important structural features are reproduced by the calculations. Furthermore, the conformational similarity of **1** and **2** implies that ethanoretinal **2** could be a good model compound of retinal **1**.

A binding experiment of **2** with aporetinochrome isolated from a squid eye according to the previously reported method[20] was carried out in a 2% digitonin solution to afford the novel retinochrome analog having the absorption maximum at 498 nm. The PSB of **2** with *n*-butylamine was prepared by the usual method. The absorption maxima, opsin shifts and CD data of artificial pigment and native retinochrome are shown in Table 2. All the values of the new retinochrome analog are very

(13) Imamoto, T.; Takiyama, N.; Nakamura, K. *Tetrahedron Lett.* **1985**, *26*, 4763. Imamoto, T.; Kusumoto, T.; Tawarayama, Y.; Sugiura, Y.; Mita, T.; Hatanaka, Y.; Yokoyama, M. *J. Org. Chem.* **1984**, *49*, 3904. Imamoto, T.; Sugiura, Y.; Takiyama, N. *Tetrahedron Lett.* **1984**, *25*, 4233.

(14) van der Steen, R.; Biesheuvel, P. L.; Erkelens, C.; Mathies, R. A.; Lugtenberg, J. *Recl. Trav. Chim. Pays-Bas* **1989**, *103*, 83.

(15) Hasbrouck, R. W.; Kiessling, A. D. A. *J. Org. Chem.* **1973**, *38*, 2103. Swaminathan, S.; Narayanan, K. V. *Chem. Rev.* **1971**, *71*, 429.

(16) Gedye, R. N.; Westaway, K. C.; Arora, P.; Bisson, R.; Khalil, A. H. *Can. J. Chem.* **1977**, *55*, 1218.

(17) For related conformational studies of retinoids using a computer program, see: Rowan, R., III; Warshel, A.; Sykes, B. D.; Karplus, M. *Biochemistry* **1974**, *13*, 970. Liu, R. S. H.; Mirzadegan, T. *J. Am. Chem. Soc.* **1988**, *110*, 8617. Asato, A. E.; Zhang, B.-Z.; Denny, M.; Mirzadegan, T.; Seff, K., Liu, R. S. H. *Bioorg. Chem.* **1989**, *17*, 410.

(18) Molecular mechanics calculations were carried out with IBM PC/2 using MMX program (Serena Software).

(19) Hamanaka, T.; Mitsui, T.; Ashida, T.; Kakudo, M. *Acta Crystallogr., Sect. B* **1978**, *28*, 214.

(20) Hara, T.; Hara, R. *Methods Enzymol.* **1982**, *81*, 190.

Table 1. Selected Structure[a] Parameters of 2 and 1

	8,18-ethanoretinal (2)	retinal (1)	
	MMX	MMX	X-ray
	(a) Bond angles (deg)		
1–2–3	111.7	111.5	115.8
2–3–4	109.5	109.2	115.6
3–4–5	113.9	114.4	115.2
4–5–6	122.9	122.4	122.6
5–6–7	119.0	121.0	122.2
6–5–18	119.9	122.7	124.3
6–7–8	122.9	122.5	124.3
7–8–9	119.9	124.9	126.3
8–9–10	121.2	118.1	118.2
9–10–11	124.9	125.2	127.1
10–9–19	120.4	121.1	123.4
10–11–12	121.5	121.6	123.4
11–12–13	125.0	125.4	125.6
12–13–14	117.7	117.7	118.2
13–14–15	124.9	124.9	125.9
	(b) Torsional angles[b] (deg)		
6–1–2–3	−49.1	−49.0	−42.6
1–2–3–4	61.0	61.1	50.0
2–3–4–5	−43.3	−44.3	−31.8
3–4–5–6	16.8	17.7	6.8
4–5–6–7	175.3	175.1	178.6
5–6–7–8	−48.3	−58.1	−58.3
6–7–8–9	174.8	179.2	−179.3
7–8–9–10	145.9	167.9	175.8
8–9–10–11	178.3	−177.4	179.1
9–10–11–12	−175.1	−178.2	−179.9
10–11–12–13	−179.8	−178.5	175.5
11–12–13–14	−168.5	−170.7	−178.4
12–13–14–15	176.8	177.3	177.7

[a] The numbering of the atom is according to the retinal molecule. [b] The sign of the torsional angle is positive in the case of clockwise.

close to those of native retinochrome. These results suggest that the conformations of both chromophores are fairly similar in the protein.

An important conclusion which results from the experimental and theoretical works reported herein is that the two conformations of **2** and **1** are similar. This suggests that the range of torsional angles around the cyclohexene ring and the polyene side chain in retinochrome could correspond to the acceptable range of 8,-18-ethanoretinal (**2**). Further work is now in progress to study the torsional angle around 6–7 bond as compared to those of another synthetic retinals.

Experimental Section

Boiling points are uncorrected. UV–vis spectra were recorded in ethanol, IR spectra in chloroform, and ^{1}H-NMR spectra in deuteriochloroform unless otherwise stated at 200 MHz or 500 MHz. Analytical HPLC was carried out with a column, LiChrosorb Si-60 (5 μm), 0.4 × 30 cm, and preparative HPLC with a LiChrosorb Si-60 (5 μm), 1.0 × 30 cm. Column chromatography (CC) under reduced pressure was performed by using Merck silica gel 60. All reactions were carried out under a nitrogen atmosphere. THF and ether were purified by distillation from benzophenone–sodium ketyl under nitrogen. Standard workup means that the organic layers were finally washed with brine, dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and concentrated *in vacuo* below 30 °C using a rotary evaporator.

Methyl 3,3-Dimethyl-2-oxocyclohexanecarboxylate (8). To a suspension of NaH (28.6 g, 0.7 mol) and dimethyl carbonate (40.2 mL, 0.47 mol) in benzene (240 mL) was added dropwise a solution of 2,2-dimethylcyclohexanone (**7**) (30 g, 0.24 mol) in benzene (240 mL) under reflux. The resulting mixture was refluxed for an additional 22 h. After cooling, the mixture was poured into ice–water (400 mL) and was made acidic by using 10% HCl. The organics were extracted with ether (3 × 200 mL) followed by standard workup. The residue was distilled (bp 68–70 °C/4 mmHg) to afford the β-keto ester **8** (34 g, 78%) as a colorless oil: UV–vis 258 nm; IR 1740, 1710, 1644, 1610 cm^{-1}; ^{1}H-NMR (200 MHz) δ 1.18 (s, 6H), 1.3–1.8 (m, 5H), 2.0–2.4 (m, 2H), 3.68 (s, 3H); exact mass calcd for $C_{10}H_{16}O_3$ 184.1099, found 184.1103 (M).

6-[5,5-(Ethylenedioxy)hexyl]-2,2-dimethylcyclohexanone (9). To a suspension of NaH (120 mg, 3.1 mmol) in THF (13.5 mL) and DMF (1.5 mL) was added a solution of β-keto ester **8** (500 mg, 2.7 mmol) in THF (15 mL) at 0 °C. After the solution was stirred for 15 min at room temperature, a solution of 1-bromo-5,5-(ethylenedioxy)hexane[8] (850 mg, 3.6 mmol) in THF (15 mL) was added dropwise, and the resulting mixture was heated under reflux for 20 h. After cooling, the mixture was poured into 10% HCl (10 mL) and the organics were extracted with ether (2 × 15 mL) followed by standard workup. The residue was purified by CC (Et_2O:hexane, 1:2) to give the ketal keto ester (599 mg, 73%) as a colorless oil: IR 1727, 1700 cm^{-1}; ^{1}H-NMR (200 MHz) δ 1.04 (s, 3H), 1.08 (s, 3H), 1.29 (s, 3H), 1.2–1.8 (m, 11H), 1.8–2.22 (m, 3H), 2.5–2.7 (m, 1H), 3.66 (s, 3H), 3.9–4.0 (s, 4H); exact mass calcd for $C_{18}H_{31}O_5$ 327.2170, found 327.2185 (M + H).

A mixture of the ketal keto ester (100 mg, 0.3 mmol) and $MgCl_2{\cdot}6H_2O$ (311 mg, 1.5 mmol) in DMSO (3 mL) was heated at 160 °C for 4 h. After cooling, water (10 mL) was added, and the organics were extracted with ether (3 × 15 mL) followed by standard workup. The residue was purified by CC (Et_2O:hexane, 1:4) to afford the ketone **9** (36 mg, 43%) as a colorless oil: IR 1700 cm^{-1}; ^{1}H-NMR (200 MHz) δ 1.04 (s, 3H), 1.17 (s, 3H), 1.31 (s, 3H) 1.2–2.0 (m, 13H), 2.0–2.2 (m, 1H), 2.4–2.6 (m, 1H), 3.93 (br s, 4H); exact mass calcd for $C_{16}H_{28}O_3$ 268.2036, found 268.2011 (M).

Ethyl 4-(3,3-Dimethyl-2-oxocyclohexyl)butyrate (11). In the same manner described for the preparation of **9**, the keto ester **11** was prepared from **8** (44 g, 230 mmol) and ethyl 4-bromobutylate (66 g, 330 mmol). The crude product was purified by CC (Et_2O:hexane, 1:4) to give the ester **11** (18.1 g, 31%) as a colorless oil: IR 1725, 1700 cm^{-1}; ^{1}H-NMR (200 MHz) δ 1.04 (s, 3H), 1.18 (s, 3H), 1.25 (t, J = 7 Hz, 3H), 1.4–2.0 (m, 9H), 2.14 (ddt, J = 13, 6, 3 Hz, 1H), 2.30 (dt, J = 1, 8 Hz, 2H), 2.4–2.7 (m, 1H), 4.16 (q, J = 7 Hz, 2H); exact mass calcd for $C_{14}H_{24}O_3$ 240.1724, found 240.1720 (M).

Ethyl 4-[2-[(Ethoxycarbonyl)methyl]-2-hydroxy-3,3-dimethylcyclohexyl]butyrate (12). To a stirred solution of LDA, prepared from *n*-BuLi (1.6 M hexane solution, 12.6 mL, 20 mmol) and diisopropylamine (2.7 mL, 20 mmol) in THF (1.6 mL), was added a solution of ethyl acetate (1.95 mL, 20 mmol) in THF (6 mL) at −70 °C, and the resulting mixture was stirred for an additional 30 min. A solution of the keto ester **11** (503 mg, 2 mmol) in THF (16 mL) was added at −70 °C, and the mixture was further stirred for 2 h. After addition of saturated aqueous NH_4Cl (40 mL) and evaporation of the solvent, the organics were extracted with ether (3 × 30 mL) followed by standard workup. The residue was purified by CC (ether:hexane, 1:4) to give the mixture of stereoisomers of **12** (524 mg, 76%) as a colorless oil: IR 3460, 1719, 1707 cm^{-1}; ^{1}H-NMR (200 MHz) δ 0.86 (s, 3/8 × 3H), 0.92 (s, 5/8 × 6H), 1.23 (s, 3/8 × 3H), 1.28 (t, J = 7 Hz, 6H), 1.3–1.8 (m, 10H), 2.1–2.4 (m, 1H), 2.24 (t, J = 7 Hz, 2H), 2.40 (d, J = 15 Hz, 5/8 H), 2.42 (d, J = 17 Hz, 3/8H), 2.51 (d, J = 15 Hz, 5/8H), 2.53 (d, J = 17 Hz, 3/8H), 4.11 (q, J = 7 Hz, 2H), 4.18 (q, J = 7 Hz, 2H), 4.30 (s, 5/8H, disappeared with D_2O), 4.90 (s, 3/8H, disappeared with D_2O); exact mass calcd for $C_{18}H_{32}O_5$ 328.2248, found 328.2258 (M).

Ethyl 4-[2-[(Ethoxycarbonyl)methyl]-3,3-dimethyl-1-cyclohexen-1-yl]butyrate (6a) and Ethyl 4-[2-[(Ethoxycarbonyl)methylene]-3,3-dimethylcyclohexyl]butylate (6b). To a solution of the hydroxy diester **12** (2.28 g, 6.95 mmol) in pyridine (46 mL) was added thionyl chloride (2.5 mL, 34.7 mmol) at 0 °C, and the resulting mixture was stirred for 10 min. The reaction was quenched with 5% HCl (200 mL) in the ice bath, and the organics were extracted with ether (3 × 80 mL) followed by standard workup. The residue was purified by CC (ether:hexane, 1:6) to give the mixture of diester **6** (2.08 g, 97%) as a colorless oil. GC (200 °C) analysis indicated that **6a** and **6b** were present in a ratio of 2.4:1.

Table 2. Absorption Maxima, CD Data, and Opsin Shift of Retinochrome and Its Analog

			retinochromes[b]			
				CD nm (mdeg/absorption)		
chromophores	aldehydes[a] λmax/nm	PSB[a] λmax/nm	λmax/nm	α-band	β-band	opsin shifts Δν/cm^{-1}
8,18-ethanoretinal (**2**)	382	449	498	488 (+4.5)	308 (−2.7)	2200
retinal (**1**)	381	443	496	495 (+5.4)	330 (−1.4)	2400

[a] In methanol. [b] In ethanol.

Analytical samples (**6a** and **6b**) were obtained, respectively, by the further CC using the same eluent.

6a: IR 1724, 1718 cm^{-1}; ^{1}H-NMR (200 MHz) δ 0.96 (s, 6H), 1.25 (t, J = 7 Hz, 3H), 1.26 (t, J = 7 Hz, 3H), 1.5−1.8 (m, 6H), 1.9−2.1 (m, 3H), 2.2−2.3 (m, 3H), 3.04 (s, 2H), 4.11 (q, J = 7 Hz, 2H), 4.12 (q, J = 7 Hz, 2H); exact mass calcd for $C_{18}H_{30}O_4$ 310.2142, found 310.2115 (M).

6b: UV 236 nm; IR 1722, 1705, 1620 cm^{-1}; ^{1}H-NMR (200 MHz) δ 1.12 (s, 3H), 1.13 (s, 3H), 1.25 (t, J = 7 Hz, 3H), 1.28 (t, J = 7 Hz, 3H), 1.4−1.9 (m, 10H), 2.36 (m, 2H), 3.7−4.0 (m, 1H), 4.12 (q, J = 7 Hz, 2H), 4.14 (q, J = 7 Hz, 2H), 5.77 (s, 1H); exact mass calcd for $C_{18}H_{31}O_4$ 311.2220, found 311.2201 (M + H).

1,1-Dimethyl-1,2,3,4,6,7,8,9-octahydro-5*H*-benzocyclohepten-8-one (5) and 1,1-Dimethyl-2,3,4,4a,5,6,7,8-octahydro-1*H*-benzocyclohepten-8-one (14). A solution of the diester **6** (2.01 g, 6.48 mmol) in xylene (50 mL) was added dropwise to a stirred suspension of *t*-BuOK (3.6 g, 32 mmol) in xylene (300 mL) at 160 °C. The mixture was further stirred for 5 min at this temperature and then cooled to room temperature. Saturated aqueous NH_4Cl (100 mL) was added, and the organic layer was separated. The aqueous layer was extracted with ether (2 × 80 mL), and the combined organic extracts were treated as standard workup. The residue was purified by CC (ether:hexane, 1:7) to give the isomeric mixture of the β-keto esters (1.31 g, 76%) as a colorless oil.

The mixture of β-keto esters (1.31 g) and $MgCl_2{\cdot}6H_2O$ (5 g, 5 equiv) in DMSO (180 mL) was heated at 160 °C for 1.5 h. After cooling, H_2O (250 mL) was added and the organics were extracted with ether (3 × 80 mL), followed by standard workup. The residue was purified by CC (ether:hexane, 1:6) to afford the bicyclic ketones **5** (933 mg, 98%) and **14** (19 mg, 2%) as colorless oils, respectively.

5: IR 1692 cm^{-1}; ^{1}H-NMR (200 MHz) δ 0.96 (s, 6H), 1.4−1.6 (m, 4H), 1.87 (t, J = 6.5 Hz, 2H), 1.98 (br, t, J = 7 Hz, 2H), 2.1−2.3 (m, 2H), 2.49 (t, J = 7 Hz, 2H), 3.12 (br s, 2H); exact mass calcd for $C_{13}H_{20}O$ 192.1512, found 192.1506 (M).

14: UV 240 nm; IR 1660, 1632 cm^{-1}; ^{1}H-NMR (200 MHz) δ 1.13 (s, 3H), 1.15 (s, 3H), 1.4−2.0 (m, 10H), 2.61 (dt, J = 2, 6 Hz, 2H), 5.94 (d, J = 2 Hz, 1H); exact mass calcd for $C_{13}H_{20}O$ 192.1528, found 192.1514 (M).

8-Hydroxy-1,1-dimethyl-8-[2-(trimethylsilyl)ethynyl]-1,2,3,4,6,7,8,9-octahydro-5*H*-benzocycloheptene (15). To a stirred suspension of anhydrous cerium chloride[13] (3.9 g, 16 mmol) in THF (57 mL) was added dropwise at −70 °C a solution of lithium (trimethylsilyl)acetylene, prepared from (trimethylsilyl)acetylene (2.2 mL, 16 mmol) and *n*-BuLi (1.6 M hexane solution, 9.6 mL, 16 mmol) in THF (25 mL). After stirring was continued for 1 h, a solution of **5** (1.19 g, 6 mmol) in THF (25 mL) was added, and the resulting mixture was stirred for an additional 3 h at −70 °C. The reaction mixture was quenched with saturated NH_4Cl (50 mL) and filtered through Celite. The filtrate was extracted with ether (3 × 80 mL) followed by standard workup. The residue was purified by CC (ether:hexane, 1:6) to afford the acetylenic alcohol **15** (1.81 g, 98%) as a colorless oil: IR 3600, 2260 cm^{-1}; ^{1}H-NMR (200 MHz) δ 0.14 (s, 9H), 0.95 (s, 3H), 1.07 (s, 3H), 1.4−1.5 (m, 2H), 1.5−1.7 (m, 4H), 1.9−2.1 (m, 6H), 2.03 (s, 1H, disappeared with D_2O), 2.53 (s, 2H); exact mass calcd for $C_{18}H_{30}OSi$ 290.2064, found 290.2054 (M).

8-Acetyl-1,1-dimethyl-1,2,3,4,6,7-hexahydro-5*H*-benzocycloheptene (3). A mixture of the acetylenic alcohol **15** (100 mg, 0.34 mmol) and 85% HCOOH (0.6 mL) was heated at 90 °C for 8 h. After cooling, the reaction mixture was neutralized with cold 5% NaOH in an ice bath. The organics were extracted with ether (3 × 20 mL) followed by standard workup. The residue was purified by CC (ether:hexane, 5:95) to afford the β-ionone analog **3** (49 mg, 65%) as a colorless oil: UV−vis 300, 236 nm; IR 1652, 1590 cm^{-1}; ^{1}H-NMR (500 MHz) δ 1.05 (s, 6H), 1.48−1.51 (m, 2H), 1.65−1.71 (m, 2H), 1.89 (t, J = 7 Hz, 2H), 2.05 (quint, J = 7.5 Hz, 2H), 2.21 (br t, J = 7 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 2.39 (s, 3H), 7.20 (br, s, 1H); exact mass calcd for $C_{15}H_{22}O$ 218.1670, found 218.1679 (M).

(2*E*)-3-(1,1-Dimethyl-1,2,3,4,6,7-hexahydro-5*H*-benzocyclohepten-8-yl)-2-butenenitrile (18). To a stirred suspension of NaH (50 mg, 1.25 mmol) in THF (2 mL) was added a solution of diisopropyl (cyanomethyl)phosphonate (188 mg, 0.92 mmol) in THF (1 mL) at room temperature. After the mixture was stirred for 30 min, a solution of the β-ionone analog **3** (105 mg, 0.48 mmol) in THF (5 mL) was added slowly at 0 °C, and the mixture was stirred for 5.5 h at room temperature. The reaction was quenched with saturated NH_4Cl (15 mL), and the organics were extracted with ether (3 × 20 mL) followed by standard workup. The residue was purified by CC (ether:hexane, 1:9) to provide the nitrile **18** (67.7 mg, 59%) as a pale yellow oil and the recovered starting compound **3** (10.9 mg, 10%).

18: UV−vis 314, 263 nm; IR 2203, 1579 cm^{-1}; ^{1}H-NMR (200 MHz) δ 0.85 (s, 6H), 1.3−1.6 (m, 4H), 1.75 (t, J = 6.5 Hz, 2H), 1.8−2.1 (m, 6H), 2.39 (s, 3H), 5.92 (s, 1H), 6.49 (s, 1H); exact mass calcd for $C_{17}H_{23}N$ 241.1830, found 241.1835 (M).

Ethyl (2*E*)-3-(1,1-Dimethyl-1,2,3,4,6,7-hexahydro-5*H*-benzocyclohepten-8-yl)-2-butenoate (16). To a stirred suspension of NaH (120 mg, 3 mmol) in THF−DMF (7:1, 2.8 mL) was added triethyl phosphonoacetate (674 mg, 3 mmol) in THF (5 mL) at 0 °C. After the mixture was stirred for 10 min, a solution of **3** (328 mg, 1.5 mmol) in THF (2 mL) was added slowly at 0 °C, and then the mixture was heated under reflux for 8 h. After cooling, the reaction was quenched with saturated NH_4Cl (20 mL), and the organics were extracted with ether (3 × 30 mL) followed by standard workup. The residue was purified by CC (ether:hexane, 5:95) to provide the ester **16** (276 mg, 64%) as a pale yellow oil and the recovered starting compound **3** (69 mg, 21%).

16: UV−vis 309, 260 nm; IR 1703, 1604 cm^{-1}; ^{1}H-NMR (500 MHz) δ 1.01 (s, 6H), 1.30 (t, J = 7 Hz, 3H), 1.46−1.50 (m, 2H), 1.64−1.70 (m, 2H), 1.87 (t, J = 7.5 Hz, 2H), 2.10 (quint, J = 7.5 Hz, 2H), 2.17 (dt, J = 1.5, 7.5 Hz, 2H), 2.21 (t, J = 7 Hz, 2H), 2.39 (d, J = 1 Hz, 3H), 4.18 (q, J = 7 Hz, 2H), 5.92 (d, J = 1 Hz, 1H), 6.48 (t, J = 1.5 Hz, 1H); exact mass calcd for $C_{19}H_{28}O_2$ 288.2087, found 288.2079 (M).

(2*E*)-3-(1,1-Dimethyl-1,2,3,4,6,7-hexahydro-5*H*-benzocyclohepten-8-yl)-2-butenal (17). (i) A solution of the ester **16** (375 mg, 1.3 mmol) in ether (10 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (100 mg, 2.6 mmol) in ether (5 mL) at 0 °C, and the resulting mixture was stirred at 0 °C for 15 min. The excess $LiAlH_4$ was destroyed by addition of moist ether and water, and then the organics were extracted with ether (3 × 30 mL) followed by standard workup. To the residue were added active MnO_2 (2.1 g, 24 mmol) and CH_2Cl_2 (12 mL), and the resulting mixture was shaken at room temperature for 3.5 h. The mixture was filtered through Celite, and the filtrate was concentrated *in vacuo* to give the crude product, which was purified by CC (Et_2O:hexane, 1:3) to afford the aldehyde **17** (180 mg, 79%) as a yellow oil: UV−vis 332, 280 nm; IR 1658, 1643, 1586, 1576 cm^{-1}; ^{1}H-NMR (200 MHz) δ 1.02 (s, 6H), 1.5−1.8 (m, 4H), 1.89 (s, J = 7.5 Hz, 2H), 2.10−2.4 (m, 6H), 2.41 (d, J = 1 Hz, 3H), 6.19 (d, J = 8 Hz, 1H), 6.73 (br s, 1H), 10.19 (d, J = 8 Hz, 1H); exact mass calcd for $C_{17}H_{24}O$ 244.1825, found 244.1820.

(ii) To a solution of the nitrile **18** (65.3 mg, 0.3 mmol) in THF (3 mL) was added dropwise diisobutylaluminum hydride

(DIBAL) (0.07 mL, 0.4 mmol) in dry THF (3 mL) at 0 °C. After the mixture was stirred for an additional 2 h at 0 °C, the excess DIBAL was destroyed by addition of moist Et_2O and water. A cold 15% tartaric acid (0.45 mL, 0.45 mmol) was added, and the resulting mixture was stirred for 2 h at room temperature. The organics were extracted with CH_2Cl_2 (3 × 5 mL) followed by standard workup. The residue was purified by CC (ether:hexane, 1:3) to afford the trienal **17** (39.4 mg, 60%) as a yellow oil. This sample was identical with an authentic species obtained by method i in all respects.

***(all-E)*-8,18-Ethanoretinal (2) and (13Z)-8,18-Ethanoretinal (21).** To a solution of diethyl 3-(methoxycarbonyl)-2-methyl-2-propenylphosphonate[16] (*E*:*Z* = 1:1) (500 mg, 2 mmol) in THF (5.5 mL) was added *n*-BuLi (1.6 M hexane solution, 0.99 mL, 1.66 mmol) at −70 °C. After the mixture was stirred for 30 min, a solution of the aldehyde **17** (162 mg, 0.66 mmol) in THF (5.5 mL) was added. The resulting mixture was allowed to come to 0 °C and then stirred for 10 min. The reaction was quenched with saturated NH_4Cl (5 mL) and extracted with ether (3 × 10 mL) followed by standard workup. The residue was purified by CC (ether:hexane, 1:9) to give the isomeric mixture of pentaenyl esters **19** and **20** (187 mg, 83%) as a yellow oil; all-*E*-isomer **19**: ^{1}H-NMR (200 MHz) δ 0.95 (s, 6H), 1.4−1.7 (m, 4H), 1.82 (t, J = 7 Hz, 2H), 1.9−2.3 (m, 6H), 2.03 (s, 3H), 2.34 (s, 3H), 3.69 (s, 3H), 5.74 (s, 1H), 6.28 (d, J = 15 Hz, 1H), 6.31 (d, J = 12 Hz, 1H), 7.01 (dd, J = 12, 15 Hz, 1H).

In the manner described for the preparation of **17** from **16**, the mixture of **19** and **20** (116 mg, 0.34 mmol) was converted to the corresponding aldehydes **2** and **21**. The crude product was purified by CC (Et_2O:hexane, 1:9) to afford two fractions. From the first fraction, 13*Z*-isomer **21** was separated by HPLC (ether:benzene:hexane, 0.15:1:3) as an orange oil (7 mg, 7%), and all-*E*-isomer **2** was separated by HPLC (ether:hexane, 12:88) from the second fraction as an orange oil (30 mg, 28%).

2: UV−vis 384 (30,600) nm (ϵ); IR 1657, 1650, 1586 cm^{-1}; ^{1}H-NMR (500 MHz, C_6D_6) δ 1.08 (s, 6H), 1.44−1.51 (m, 2H), 1.58−1.64 (m, 2H), 1.74 (d, J = 1 Hz, 3H), 1.89 (t, J = 7 Hz, 2H), 1.90 (s, 3H), 2.03 (quint, J = 7 Hz, 2H), 2.08 (br t, J = 7 Hz, 2H), 2.30 (t, J = 7 Hz, 2H), 5.99 (br d, J = 8 Hz, 1H), 6.05 (d, J = 15 Hz, 1H), 6.35 (d, J = 11 Hz, 1H), 6.47 (br s, 1H), 6.89 (dd, J = 11, 15 Hz, 1H), 10.02 (d, J = 8 Hz, 1H); exact mass calcd for $C_{22}H_{30}O$ 310.2295, found 310.2298 (M).

21: UV−vis 382 (27,000), 268 (9,000), 228 (8,800) nm (ϵ). ^{1}H-NMR (500 MHz, C_6D_6) δ 1.09 (s, 6H), 1.44−1.50 (m, 2H), 1.60−1.65 (m, 2H), 1.62 (br s, 3H), 1.91 (s, 3H) 1.91 (t, J = 7 Hz, 2H), 2.05 (quint, J = 7 Hz, 2H), 2.09 (dt, J = 1, 7 Hz, 2H), 2.32 (t, J = 7.5 Hz, 2H), 5.77 (br d, J = 7.5 Hz, 1H), 6.44 (d, J = 12 Hz, 1H), 6.49 (br s, 1H), 6.83 (dd, J = 12, 14.5 Hz, 1H), 7.10 (d, J = 14.5 Hz, 1H), 10.11 (d, J = 7.5 Hz, 1H); exact mass calcd for $C_{22}H_{30}O$ 310.2295, found 310.2294 (M).

Preparation of Analog Pigment. All manipulations were performed in the dark or dim red light. Retinochrome was extracted from the squid retinas (*Todarodes pacificus*) with a 2% digitonin solution in 67 mM phosphate buffer[20] (pH 6.5). Aporetinochrome was prepared by irradiation of retinochrome with light (>350 nm) using a 500 W Xe lamp. For the formation of artificial retinochrome, an excess of the (*all-E*)-8,18-ethanoretinal (**2**) (2- to 3-fold) was mixed with a solution of aporetinochrome 2% digitonin in phosphate buffer (*ca.* 15 μM). In order to obtain genuine spectra, the mixture was washed three times with hexane to remove excess of analog **2**. The PSB of the retinal analog **2** was formed by adding several equivalents of butylamine in methanol to a methanolic solution of all-*E* analog **2** (*ca.* 20 μM) followed by titrating with a dry HCl methanolic solution until the solution showed no change in the spectra.

Supplementary Material Available: ^{1}H-NMR spectra for compounds **2**, **3**, **5**, **6a**, **6b**, **11**, **12**, **15-18**, and **21** (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.