Retinoids and related compounds. Part 20.[1] Synthesis of (11*Z*)-8,18-ethanoretinal and a conformational study of the rhodopsin chromophore

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In order to investigate the conformation of the chromophore around the trimethylcyclohexene ring in rhodopsin, (11*Z*)-8,18-ethanoretinal 6 has been synthesized. Its binding experiment with bovine opsin afforded a new rhodopsin analogue, the opsin shift and CD spectrum of which were similar to those of natural rhodopsin. These experimental results strongly suggest that the torsional angle around the 6–7 single bond in rhodopsin corresponds to the torsional angle in (11*Z*)-8,18-ethanoretinal 6. In addition, MMX calculations for 2 and 6 have been carried out and are reported.

Rhodopsin (Rh) **1** is the visual pigment which contains (11*Z*)-retinal **2** as a chromophore bound to the ε-amino group of the apoprotein lysine residue through a protonated Schiff base (PSB) (Fig. 1).[2] Although both (11*Z*)-retinal and apoprotein opsin show no optical activity in the visible (VIS) and near-UV region, rhodopsin exhibits characteristic circular dichroic (CD) absorption as α and β bands [α-band: 487 nm (+7.5), β-band: 335 nm (+15.4)].[3] To elucidate the molecular mechanism leading to the generation of the specific CD spectrum in rhodopsin, we have synthesized various retinal analogues and confirmed that the α-CD band originates from the torsion around the 12–13 single bond of the chromophore. That is, the Rh analogue **3** having a non-twisted conformation at this bond because of its fixation by the 5-membered ring, shows no CD absorption corresponding to an α-band [β-band: 336 nm (+11.6)]. On the other hand, CD absorption data for the bicyclic Rh analogues **4**[4f] [α-band: 512 nm (+13.6), β-band: 326 nm (−2.1)] and **5**[4g] [α-band: 526 nm (+12.3), β-band: 332 nm (+31.0)], having 6s-*cis* fixed 9*Z*- and 11*Z*-chromophores suggests that the twisted 6s-single bond strongly affects the β-band of Rh. In order to clarify the torsional angle around the cyclohexene ring and polyene side-chain in the chromophore, we describe here both a full account of the synthesis of (11*Z*)-8,18-ethanoretinal **6**, in which the C-8 and C-18 positions in **2** are connected by an ethylene group, and its interaction with bovine opsin; this work has been the subject of an earlier communication.[5]

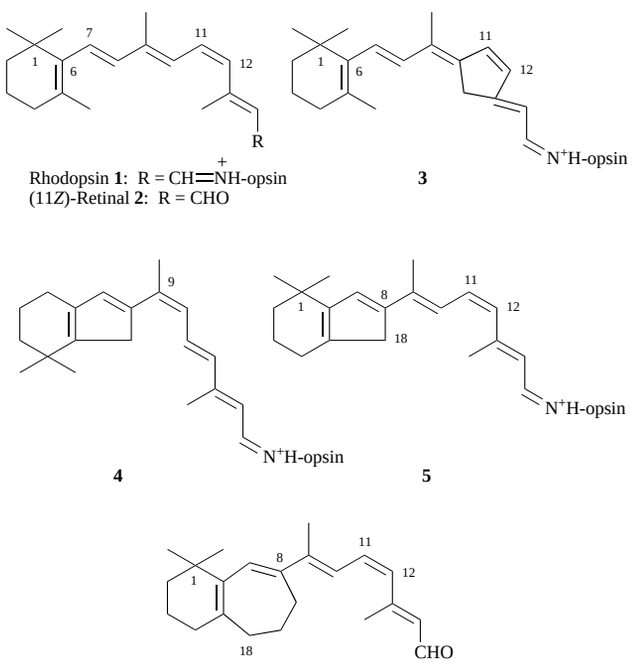

Fig. 1

Results and discussion

It is well known that photochemical isomerization is the most convenient method for obtaining (11*Z*)-retinal and its analogues.[6] Since we had recently synthesized (all-*E*)-8,18-ethanoretinal **9** by condensation of the aldehyde **8** and a C-5-phosphonate as a key reaction, and allowed it to react with *apo*-retinochrome,[7] we decided to photoisomerize compound **9**. Thus, irradiation of a methanol or acetonitrile solution of **9** under nitrogen using a 40 W fluorescent daylight lamp afforded an isomeric double-bond mixture. The all-*E*- and 9*Z*-isomers, **9** and **10**, were isolated in pure form after separation using high performance liquid chromatography (HPLC). The structure of the 9*Z*-isomer **10** was determined by comparison of its ^{1}H NMR spectral results with those of other isomers and the corresponding retinal isomers (Table 1).

Kobayashi *et al.*[8] reported a high yield synthesis of the retinal analogue containing the 11*Z*-isomer by a Wittig reaction of the β-ionylideneacetaldehyde analogue with 3-hydroxymethyl-2-methylallyl(triphenyl)phosphonium hydrobromide under

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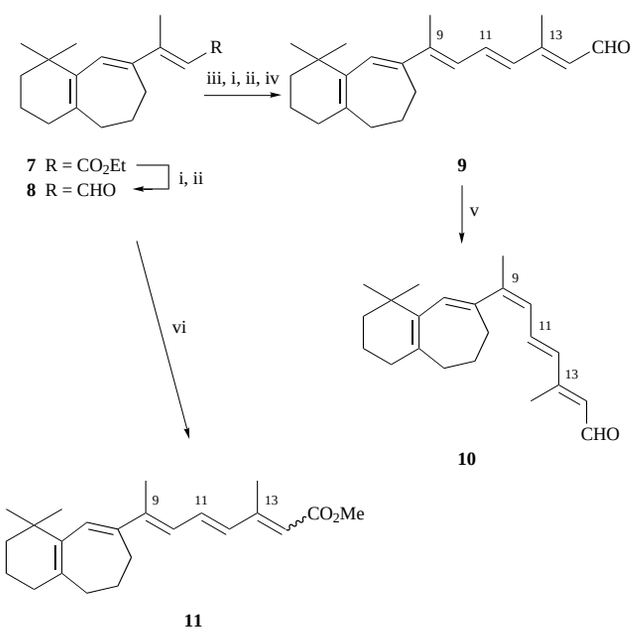
Table 1 ^{1}H NMR chemical shifts of 8,18-ethanoretinals and retinals[a]

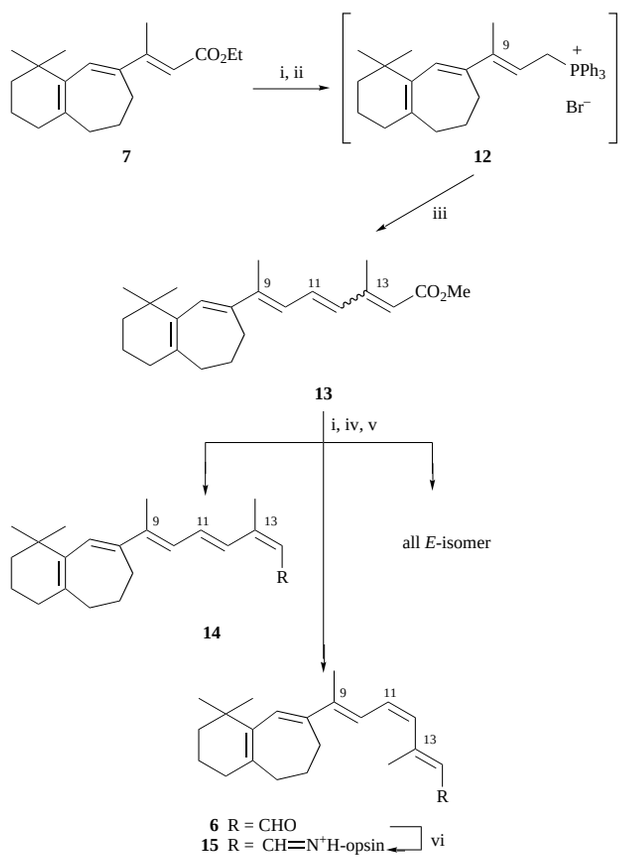
		All-*E* **9**[7]	9*Z* **10**	11*Z* **6**	13*Z* **14**[7]
^{1}H NMR	1-Me	1.08 (s)	1.03 (s)	1.07 (s)	1.09 (s)
(500 MHz)	9-Me	1.90 (s)	1.82 (s)	1.86 (s)	1.91 (s)
(δ, C_6D_6)	13-Me	1.74 (d, *J* = 1)	1.80 (d, *J* = 1)	1.80 (d, *J* = 1)	1.62 (br s)
	7-H	6.47 (br s)	6.08 (br s)	6.45 (br s)	6.49 (br s)
	10-H	6.35 (d, *J* = 11)	5.84 (d, *J* = 11)	6.90 (d, *J* = 12)	6.44 (d, *J* = 12)
	11-H	6.89 (dd, *J* = 11, 15)	7.11 (dd, *J* = 11, 16)	6.47 (br t, *J* = 12)	6.83 (dd, *J* = 12, 14.5)
	12-H	6.05 (d, *J* = 15)	6.05 (d, *J* = 16)	5.63 (d, *J* = 12)	7.10 (d, *J* = 14.5)
	14-H	5.99 (d, *J* = 8)	5.99 (d, *J* = 8)	6.16 (d, *J* = 8)	5.77 (d, *J* = 7.5)
	CHO	10.07 (d, *J* = 8)	9.99 (d, *J* = 8)	9.95 (d, *J* = 8)	10.11 (d, *J* = 7.5)
		All-*E*-retinal	9*Z*-retinal	11*Z*-retinal	13*Z*-retinal
^{1}H NMR	1-Me	1.12 (s)	1.09 (s)	1.07 (s)	1.13 (s)
(500 MHz)	9-Me	1.78 (d, *J* = 0.5)	1.86 (s)	1.74 (d, *J* = 1)	1.78 (s)
(δ, C_6D_6)	13-Me	1.74 (d, *J* = 1.5)	1.62 (d, *J* = 1)	1.76 (d, *J* = 1.5)	1.59 (d, *J* = 1)
	7-H	6.36 (d, *J* = 16)	6.37 (d, *J* = 16)	6.34 (d, *J* = 16)	6.37 (d, *J* = 16)
	8-H	6.26 (d, *J* = 16)	6.87 (d, *J* = 16)	6.23 (d, *J* = 16)	6.28 (d, *J* = 16)
	10-H	6.02 (d, *J* = 11.5)	5.89 (d, *J* = 11.5)	6.59 (d, *J* = 12)	6.05 (d, *J* = 11.5)
	11-H	6.84 (dd, *J* = 11.5, 16)	7.07 (dd, *J* = 11.5, 16)	6.38 (t, *J* = 12)	6.74 (dd, *J* = 11.5, 15)
	12-H	6.04 (d, *J* = 16)	5.97 (d, *J* = 16)	5.59 (d, *J* = 12)	7.07 (d, *J* = 15)
	14-H	5.96 (d, *J* = 8)	5.94 (d, *J* = 8)	6.11 (d, *J* = 7.5)	5.75 (d, *J* = 7.5)
	CHO	10.02 (d, *J* = 8)	9.95 (d, *J* = 8)	9.91 (d, *J* = 7.5)	10.15 (d, *J* = 7.5)

[a] *J* values are given in Hz.



7 **9** **10** **11**

Scheme 1 *Reagents and conditions:* i, LAH, Et_2O, 0 °C; ii, MnO_2, CH_2Cl_2, 38% from **7**; iii, BuLi, $(EtO)_2P(O)CH_2C(Me)=CHCO_2Me$, THF, 0 °C, 44%; iv, preparative HPLC; v, *hv*, MeCN; vi, NaOMe, $Ph_3P=CHC(Me)=CHCO_2Me$, C_6H_6



7 **12** **13** **14** **6** **15**

Scheme 2 *Reagents and conditions:* i, LAH, Et_2O, 0 °C; ii, $Ph_3P{\cdot}HBr$, MeOH; iii, NaOMe, $OHCC(Me)=CHCO_2Me$, CH_2Cl_2, 0 °C, 44%; iv, MnO_2, CH_2Cl_2, 38% from **13**; v, preparative HPLC; vi, bovine opsin

basic conditions and subsequent MnO_2 oxidation. Application of this method with minor modifications to the aldehyde **8** and 3-methoxycarbonyl-2-methylallyl(triphenyl)phosphorane gave a mixture of 13,14 double-bond geometrical isomers **11**, none of the 11*Z*-isomer **6** being detected. From this we conclude that use of a stabilized phosphorane in the Wittig reaction precludes formation of the 11*Z*-isomer **6**.

Since in earlier work on the synthesis of retinoid analogues, we prepared the 11*Z*-isomer[4a,g] from a Wittig reaction between β-ionylideneethyl(triphenyl)phosphonium salt and the C-5 aldehyde, we used this methodology as follows. The reaction of methyl (*E*)-3-formylcrotonate with the phosphonium salt **12**, which was derived from the ester **7** by $LiAlH_4$ (LAH) reduction followed by treatment with triphenylphosphonium hydrobromide, in the presence of sodium methoxide afforded the pentaenyl ester **13** as a mixture of geometrical isomers (all-*E*:11*Z* = *ca.* 2:1). Without isolation of these isomers, the transformation of **13** to the corresponding aldehyde mixture (**6**, **9** and **14**) was achieved by $LiAlH_4$ reduction followed by MnO_2 oxidation. The 11*Z*-isomer **6** was isolated by repeated preparative HPLC separation in the dark. The structure of **6** was assigned on the basis of ^{1}H NMR spectral results, which exhibited a triplet for the 11-H signal at δ 6.47. The assignment of all signals was carried out by the comparison of chemical-shift values with those of (11*Z*)-retinal **2** (Table 1).

(11*Z*)-8,18-Ethanoretinal **6** showed an absorption maximum at 386 nm (EtOH), which is very close to that of (11*Z*)-retinal

Table 2 Selected structural[a] parameters of **6** and **2**

	(11*Z*)-Ethanoretinal **6**	(11*Z*)-Retinal **2**		(11*Z*)-12s-*cis*-Retinal **2**
	MMX	MMX	X-ray	MMX
(a) Bond angles (°)				
1, 2, 3	111.6	111.5	111.5	111.5
2, 3, 4	109.6	109.4	111.9	109.3
3, 4, 5	114.0	114.6	113.3	114.5
4, 5, 6	122.8	122.4	122.9	122.4
5, 6, 7	119.2	121.2	123.2	121.0
6, 5, 18	119.8	122.7	125.8	122.6
6, 7, 8	122.5	121.2	126.2	121.6
7, 8, 9	120.8	125.2	126.4	125.8
8, 9, 10	120.3	117.8	117.8	117.7
9, 10, 11	125.0	125.2	125.3	125.3
10, 9, 19	120.4	121.4	124.3	121.0
10, 11, 12	125.3	125.8	128.1	125.0
11, 12, 13	127.6	128.0	129.9	126.7
12, 13, 14	117.7	117.5	121.3	120.6
13, 14, 15	124.6	124.6	122.9	124.1
(b) Torsional angles (°)[b]				
6, 1, 2, 3	−49.2	−49.9	−47.4	−49.7
1, 2, 3, 4	61.1	60.7	59.1	60.8
2, 3, 4, 5	−42.9	−43.2	−39.8	−43.3
3, 4, 5, 6	15.8	17.2	11.5	16.9
4, 5, 6, 7	175.3	173.6	−179.6	174.1
5, 6, 7, 8	−50.1	−57.4	−41.4	−58.9
6, 7, 8, 9	−179.7	−178.3	179.6	−179.7
7, 8, 9, 10	−153.3	−166.7	174.0	170.0
8, 9, 10, 11	179.2	177.7	175.5	−178.0
9, 10, 11, 12	163.1	172.0	179.3	177.5
10, 11, 12, 13	−11.8	−10.2	−2.1	−5.9
11, 12, 13, 14	155.6	151.1	−38.7	−48.9
12, 13, 14, 15	179.7	−179.6	179.8	179.6

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

Table 3 Absorption maxima, CD data and opsin shift of rhodopsin and its analogue

			Rhodopsins[c] CD (nm)			
				(mdeg/absorption)		
Chromophores	Aldehydes[a] (λ_{max}/nm)	PSB[b] (λ_{max}/nm)	(λ_{max}/nm)	α-band	β-band	Opsin shifts ($\Delta\nu$/cm^{-1})
(11*Z*)-8,18-Ethanoretinal **6**	386	457	503	491 (+8.23)	335 (+16.97)	2000
(11*Z*)-Retinal **2**	377	440	498	489 (+8.67)	330 (+17.50)	2650

[a] In ethanol. [b] In methanol. [c] In a CHAPS–PC mixture.

2 (377 nm) and indicates the close similarities of the co-planarities in the conjugated parts of these two compounds. Molecular mechanics calculations were carried out to evaluate the structural conformation[9] of **6** and (11*Z*)-retinal **2**. Although many stable conformers are possible within a range of 1 kcal mol^{-1} steric-energy differences in **6** and **2** respectively, structural parameters of the most stable conformers of **6** (l2s-*trans*-structure) and **2** (l2s-*trans*- and l2s-*cis*-structure) and an X-ray analysis of (11*Z*)-retinal (l2s-*cis*-structure)[10] are summarized in Table 2. The good agreement between the calculated and X-ray crystallographic data of **2** is satisfactory in that most of the important structural features are reproduced by the calculations. Furthermore, the conformational similarity of **6** and **2** implies that 8,18-ethanoretinal **2** could be a good model compound for retinal **2**.

A binding experiment of **6** with bovine opsin, isolated according to a previously reported method,[11] was carried out in a 3-[(3-cholamidopropyl)dimethylammonio]propane-1-sulfonate–phosphatidylcholine (CHAPS–PC) mixture to afford the novel rhodopsin analogue **15** having an absorption maximum at 503 nm. The PSB of **6** with butylamine was formed by the usual method. The absorption maxima, opsin shifts and CD data of the artificial pigment and native rhodopsin are shown in Table 3. All the values of the new pigment analogue **15** are very close to those of native rhodopsin. These results suggest that the conformations of the two chromophores in the protein are closely similar.

An important conclusion obtained from the experimental and theoretical work reported here is that the two conformations of **6** and **2** are very similar. In particular, it is suggested that the range of the torsional angle around the cyclohexene ring and the polyene side-chain in rhodopsin corresponds to that for 8,18-ethanoretinal **6**. Further work is now in progress to study the torsional angle around the 6–7 bond as compared with those of other synthetic retinals.

Experimental

Ether refers to diethyl ether. BuLi was used as a solution in hexane. UV–VIS spectra were recorded on a JASCO Ubest-55 instrument and IR or FT-IR spectra on a Shimadzu IR-27G or Shimadzu FT-IR-4200 spectrometer. ^{1}H NMR spectra at 500 MHz were measured on a Varian VXR-500 superconducting FT-NMR spectrometer using tetramethylsilane as an internal reference. ^{13}C NMR spectra were recorded on a Varian VXR-500 instrument operating at 125 MHz. Mass spectra were

determined on a Hitachi M-4100 spectrometer. Preparative HPLC was conducted on a Shimadzu LC-6A instrument with a Shimadzu UV–VIS detector, SPD-6AV, using a LiChrosorb Si-60 (5 μm), 1.0 × 30 cm column. Unless otherwise stated, solvent extracts were dried over anhydrous sodium sulfate and all operations were carried out under nitrogen or argon. The extract or the filtrate was concentrated under reduced pressure at <30 °C using a rotary evaporator. Molecular mechanics calculations were carried out with an IBM PC/2 using the MMX program (Serena Software).

Photoisomerization of (all-*E*)-8,18-ethanoretinal 9

All-*E* bicyclic retinal **9**[7] (5 mg, 0.016 mmol) in MeCN (7 cm^3) was irradiated with a daylight fluorescent lamp (40 W, without filter) at room temp. for 5 h to give a mixture of geometrical isomers. After removal of the solvent from the reaction mixture, the residue was purified by HPLC [LiChrosorb Si-60 (5 μm), 1 × 25 cm, hexane–benzene–Et_2O (3:1:0.2), detector wavelength 370 nm, flow rate 2.8 ml min^{-1}] in the dark to afford the (all-*E*)-**9** (2.1 mg, 42%) and the 9*Z*-isomers **10** (34%).

9*Z*-Isomer **10**; λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 352 (9297); ν_{max}(KBr)/cm^{-1} 1660 and 1575; δ_H(C_6D_6) 1.03 (6H, s, 1,1-gem-Me), 1.42–1.45 (2H, m, 2-H_2), 1.54–1.62 (2H, m, 3-H_2), 1.80 (3H, d, *J* 1, 13-Me), 1.82 (3H, br s, 9-Me) 1.92–2.00 (6H, m, 18-H_2, 18a-H_2, 18b-H_2), 2.06 (2H, dt, *J* 1.5, 6.5, 4-H_2), 5.84 (1H, br d, *J* 11, 10-H), 5.99 (1H, br d, *J* 8, 14-H), 6.05 (1H, d, *J* 16, 12-H), 6.08 (1H, br s, 7-H), 7.11 (1H, dd, *J* 11, 16, 11-H) and 9.99 (1H, d, *J* 8, 15-H); δ_C(C_6D_6) 12.71 (Me), 19.98 (CH_2), 24.43 (Me), 28.96 (1,1-gem-Me), 29.97 (CH_2), 32.36 (CH_2), 33.19 (CH_2), 34.00 (C), 36.22 (CH_2), 39.20 (CH_2), 126.02 (CH), 129.42 (CH), 129.46 (CH), 133.59 (CH), 134.08 (CH), 136.67 (C), 138.64 (C), 143.91 (C), 148.10 (C), 153.49 (C) and 189.74 (CHO) [Found (HRMS): *m*/*z* 310.2300. Calc. for $C_{22}H_{30}O$: M^+, 310.2296].

Wittig reaction of the trienal 8 and phosphorane

A mixture of the aldehyde **8**[7] (96.4 mg, 0.4 mmol), 3-methoxycarbonyl-2-methylprop-2-enylidene(triphenyl)-phosphorane (600 mg, 1.6 mmol) and toluene (5 cm^3) were refluxed for 24 h. After removal of the solvent, the residue was purified by CC (Et_2O–hexane, 1:3) to give an isomeric mixture of the ester **11** (38.7 mg, 29%) and starting compound **8** (69 mg). All-*E*-isomer of **11**; δ_H($CDCl_3$) 1.01 (6H, s, 1′-Me × 2), 1.30 (3H, t, *J* 7, $CO_2CH_2CH_3$), 1.46–1.50 (2H, m, 2′-H_2), 1.64–1.70 (2H, m, 3′-H_2), 1.87 (2H, t, *J* 7.5, 5′-H_2), 2.10 (2H, quintet-like, *J* 7.5, 6′-H_2), 2.17 (2H, dt, *J* 1.5, 7.5, 4′-H_2), 2.21 (2H, t-like, *J* 7, 7′-H_2), 2.39 (3H, d, *J* 1, 3-Me), 4.18 (2H, q, *J* 7, $CO_2CH_2CH_3$), 5.92 (1H, d, *J* 1, 2-H) and 6.48 (1H, t, *J* 1.5, 9′-H).

(11*Z*)-8,18-Ethanoretinal 6

A solution of the trienyl ester **7**[7] (375 mg, 1.3 mmol) in dry Et_2O (10 cm^3) was added to a stirred suspension of LAH (100 mg, 2.6 mmol) in dry Et_2O (10 cm^3) at 0 °C under nitrogen, and the resulting mixture was stirred for an additional 15 min. The excess of LAH was destroyed by the addition of moist Et_2O and water to the mixture which was then extracted with Et_2O. The extract was washed with brine, dried and concentrated to afford the crude alcohol, which was used in the next reaction without further purification. Triphenylphosphine hydrobromide (445 mg, 1.3 mmol) was added dropwise to a stirred solution of the crude alcohol in methanol (10 cm^3), and the resulting mixture was stirred for 20 h at room temperature. Evaporation of the solvent gave a residue, which was washed with ether to provide the Wittig salt. A solution of the Wittig salt in $CHCl_3$ (8 cm^3) was added to a stirred solution of methyl 3-formylcrotonate (200 mg, 1.5 mmol) in MeOH (2 cm^3, containing NaOMe, 1.3 mmol) at 0 °C. The resulting mixture was stirred for 1 h and then diluted with water (5 cm^3). After removal of the solvent, the residue was extracted with ether. The extract was washed with brine, dried (Na_2SO_4) and evaporated to give a crude product, which was purified by CC (ether–hexane, 1:9) to afford the pentaenyl ester **13** (217 mg, 49%) as a mixture of stereoisomers.

A solution of the ester **13** (217 mg, 0.7 mmol) in dry Et_2O (5 cm^3) was added to a stirred suspension of LAH (50 mg, 1.3 mmol) in dry Et_2O at 0 °C. After the mixture had been stirred at 0 °C for 15 min, it was treated with moist Et_2O and water to destroy the excess of LAH, and extracted by Et_2O. The combined extracts were washed with brine, dried and concentrated to give the hydroxy compound as a pale yellow amorphous product. A mixture of the resulting hydroxy compound and active MnO_2 (2.1 g, 24 mmol) in dry CH_2Cl_2 (20 cm^3) was shaken at room temp. for 3 h and then filtered through Celite. Evaporation of the filtrate gave an oil which was purified by CC (Et_2O–hexane, 1:9) to yield an isomeric mixture of the aldehydes (155 mg, 58%) as an orange oil. Separation of the isomers was achieved by preparative HPLC [LiChrosorb Si-60 (5 μm) 1 × 30 cm, hexane–benzene–ether (3:1:0.2), 2.1 ml min^{-1}, 350 nm] to give the 13*Z*-isomer **14** (7.4 mg), the 11*Z*-isomer **6** (11.3 mg) and the all-*E*-isomer **9** (26 mg) respectively, in a pure state, in a ratio of 1:2:4. The spectral properties of **14** and **9** were identical with those of authentic specimens.[7]

11*Z*-Isomer **6**; λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 386 (17 935), 296 (10 368), 266 (10 942) and 230 (9860); ν_{max}(KBr)/cm^{-1} 1660 and 1575; δ_H(C_6D_6) 1.07 (6H, s, 1,1-gem-Me), 1.42–1.47 (2H, m, 2-H_2), 1.55–1.62 (2H, m, 3-H_2), 1.80 (3H, d, *J* 1, 13-Me), 1.86 (3H, br s, 9-Me), 1.88 (2H, t, *J* 7.5, 18-H_2), 2.05 (2H, quintet-like, *J* 7, 18a-H_2), 2.09 (2H, t, *J* 6.5, 4-H_2), 2.29 (2H, t, *J* 7, 18b-H_2), 5.63 (1H, br d, *J* 12, 12-H), 6.16 (1H, br d, *J* 8, 14-H), 6.45 (1H, br s, 7-H), 6.47 (1H, t-like, *J* 12, 11-H), 6.90 (1H, br d, *J* 12, 10-H) and 9.95 (1H, d, *J* 8, CHO); δ_C(C_6D_6) 14.17 (Me), 17.51 (Me), 19.99 (CH_2), 28.15 (CH_2), 29.02 (1,1-gem-Me), 32.39 (CH_2), 33.04 (CH_2), 34.24 (C), 36.24 (CH_2), 39.28 (CH_2), 122.34 (CH), 128.23 (CH), 130.65 (CH), 130.88 (CH), 131.78 (CH), 137.28 (C), 139.77 (C), 142.42 (C), 145.19 (C), 154.08 (C) and 189.76 (CHO) [Found (HRMS): *m*/*z* 310.2297. Calc. for $C_{22}H_{30}O$: M^+, 310.2296].

Binding of the retinal analogue 6 with bovine opsin

(11*Z*)-8,18-Ethanoretinal **6** (3–4 mol equiv.) dissolved in a small amount of ethanol was added to the opsin preparation and incubated at 23 °C in the dark for 40 h. The reaction mixture was then applied to DEAE-Sepharose column (Pharmacia) which had been equilibrated with buffer C {50 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), 0.6% CHAPS, 0.8 mg cm^{-3} PC, 20% (w/v) glycerol, pH 6.6}. The column was washed with buffer C supplemented with 10 mM hydroxylamine to remove the excess of retinal analogue; hydroxylamine was then removed by washing the column with buffer C. The rhodopsin analogue was eluted with buffer C supplemented with 140 mM aqueous NaCl.

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