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11-Methyl-9-demethylretinal and 11-Methyl-9,13-didemethylretinal. Effect of Altered Methyl Substitution Pattern on Polyene Conformation, Photoisomerization and Formation of Visual Pigment Analogs

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Abstract. Altered methyl substitution pattern in 11-methyl-9-demethylretinal (1) and 11-methyl-9,13-didemethylretinal (2) resulted in changed polyene conformation, regioselectivity of photoisomerization, uv/vis characteristics and reactivity toward bovine opsin. That the 11-cis isomer of 1 and 9-cis isomer of 2 gave visual pigment analogs (λ_{max} 475 and 459 nm respectively) while the 11-cis isomer of 2 did not is consistent with the requirement of a twisted chromophore near the middle of the polyene chain for pigment formation.

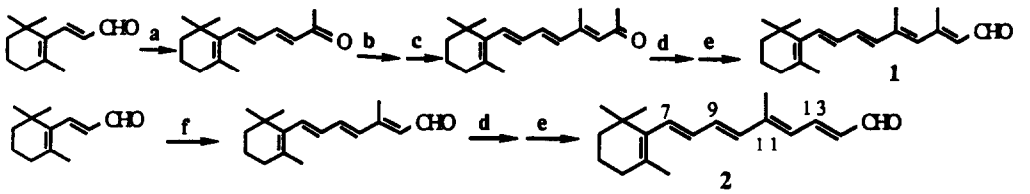
Introduction

The pattern of methyl substitution in a polyene such as retinal and its derivatives is believed to have significant effects on the polyene conformation,[1] direction of photoisomerization,[2] spectroscopic properties of some of their hindered isomers,[3] and formation of visual pigment analogs.[4] Hence, direct irradiation of a hexane solution of retinal results in regioselective isomerization at the terminal trisubstituted 13,14 or the middle 9,10 bond.[5] And, removal of either one of the methyl groups on the polyene side chain results in inefficient isomerization at that double bond.[2] Isomerization around the unsubstituted 11,12 bond takes place only in a hydrogen bonding solvent.[6] The temperature dependent spectroscopic properties of 11-*cis*-retinal is believed due to conformational changes as a result of steric interaction of the 13-methyl group with H-10.[7] And, the 12-methyl and 10-alkyl substituents have significant effects on pigment yield.[4b] In this paper we report the preparation of two new retinal analogs with a different pattern of methyl substitution: 11-methyl-9-demethylretinal, 1, and 11-methyl-9,13-didemethylretinal, 2, and their photochemical properties and results of binding interaction with bovine opsin.

Results and Discussion

Synthesis. Compounds 1 and 2 were prepared starting with the C_{12}-aldehyde following established procedures for olefination of compounds in the retinoid series (see scheme below).[8] In a separate run, the trifluoroethoxy analog of the C_5-phosphonate f[9] was used which allowed

isolation of the 9-cis isomers. These nonstereoselective sequences led to four isomers of 1 and 2. All of them were isolated by preparative hplc.



a. Acetone/NaH. b. $TMSCH_2CO_2Et$. c. KOH/MeLi. d. $(C_2H_5O)_2POCH_2CN$/NaH. e. DIBAH. f. $(C_2H_5O)_2POCH_2C(CH_3)$=CHCN/NaH

The 11,13-dicis and 13-cis isomers of 1 interconverted readily at room temperature. The situation is reminiscent of that of 11-methylretinal.[10] At 22°C, 13-*cis*-1 and 11,13-di*cis*-1 were found to be present in an equilibrium mixture of 68 and 32% with the respective rate constants of interconversion being 7.3 x 10^{-5} and 1.5 x 10^{-4} s^{-1}. These are to be compared with 2.8 and 4.8 x 10^{-4} sec^{-1} for the respective isomers of 11-methylretinal.[10]

Conformational Properties. Methyl substituents are known to affect significantly conformational properties of isomers of retinal.[7] Relocation of the 9-methyl group in retinal to the 11-position as in 1 is expected to introduce added steric crowding between the 11- and 13-methyl groups. This point is clearly revealed in a NOE experiment: irradiation of CH_3-11 of all-*trans*-1 at 22°C led to enhancement of CH_3-13, H-9 and H-14 by 2.2, 5.8 and 3.3% respectively. Similar irradiation at -85°C, however, led to enhancement of the same signals by 0, 11.9 and 13.1% respectively. The results are consistent with equilibration between the 12-S-cis and 12-S-trans conformation (probably both twisted) favoring the former. The preference for the S-cis conformation is probably the cause for the rapid interconversion of 13-*cis*-1 and 11,13-di*cis*-1.[11] Accessibility of the dicis conformers are necessary for formation of the postulated α-pyran intermediate.[12]

Temperature dependent uv-vis spectral characteristics of the 11-*cis*-retinal are believed to be due to population inversion of the 12-S-cis and 12-S-trans conformers.[3] We have examined this

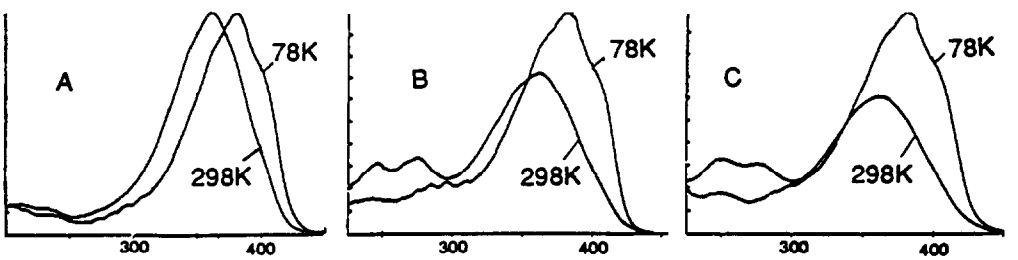


Figure 1. Temperature dependent uv/vis absorption spectra of (a) all-trans (b) 9-cis and (c) 9,13-dicis isomers of 2 taken at 298K and 78K in 3-methylpentane.

feature with the current compounds. The 11-methyl group in 1 and 2 makes their 9-cis isomers sterically equivalent to the hindered 11-cis isomer of the parent retinal. Indeed, 9-cis and 9,13-dicis isomers of 2 exhibit similar tempera-ture dependent uv-vis absorption spectra (Figure 1) as 11-*cis*-retinal[13] with the long wavelength band showing bathochromic shift and a simultaneous increase in the extinction coefficient upon cooling. Absorption near the 250 nm region, on the other hand, showed a decrease in intensity. This behavior is also consistent with the involvement of two equilibrating conformers (10-S-cis and 10-S-trans) as in the case of 11-*cis*-retinal. Consistent with this interpretation is the absence of temperature dependent behavior for the all-trans isomer of 2.

Upon addition of the 13-methyl group as in 1, spectra of the 11-cis isomer again exhibit temperature dependence (Figure 2). Interestingly, the isomer shows a much stronger "cis" band while the bathochromic shift is accompanied by a slight decrease in intensity of the long

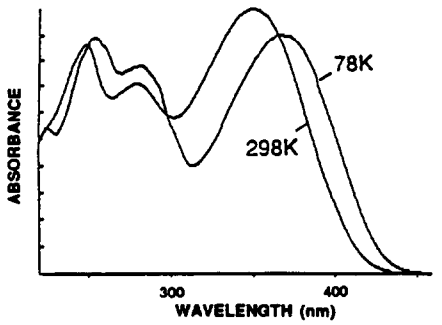


Figure 2. Temperature dependent uv/vis absorption spectra of 11-cis-1 in 3-methylpentane.

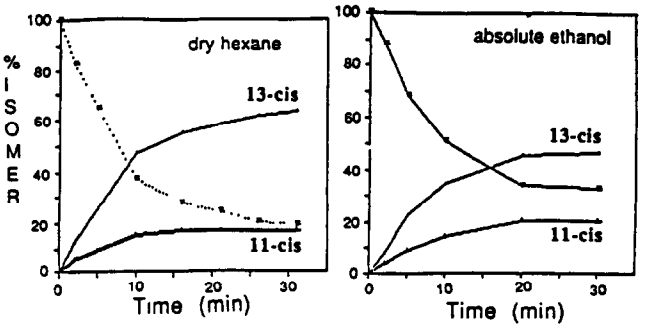
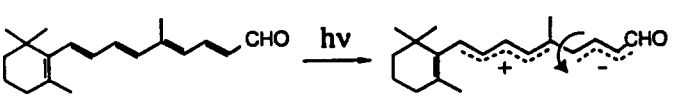


Figure 3. Progress of the isomerization reaction from direct irradiation of the all-trans isomer of 1 in two different solvents.

wavelength band. While we do not have a definitive explanation for the altered temperature dependent behavior as shown in the uv/vis spectrum of 11-*cis*-1, preliminary MMP2-85 calculation suggests possible altered conformational preference around the 6,7 bond relative to the 12,13 bond upon relocation of the 9-methyl group while retaining the 13-methyl group.[14]

Photoisomerization. Photoisomerization under direct irradiation of the all-trans isomer of 1 and the all-trans and 11-cis isomers of 2 were studied. Reaction progress is depicted in Figures 3 and 4. Photostationary state compositions obtained from these studies are summarized in Table 1 along with those reported for all-*trans*-retinal.[6b]

It is clear that the photochemical behavior of trans isomers of 1 and 2 are different from that of retinal. The presence of the 11-methyl group makes the 11-cis isomer a major photoproduct regardless of the choice of solvent (hexane or ethanol). This is possibly a reflection of the electron donating character of the methyl substituent stabilizing the dipolar character[15] of the excited singlet state of retinal (polyenals).[2b] For the parent retinal, isomerizing



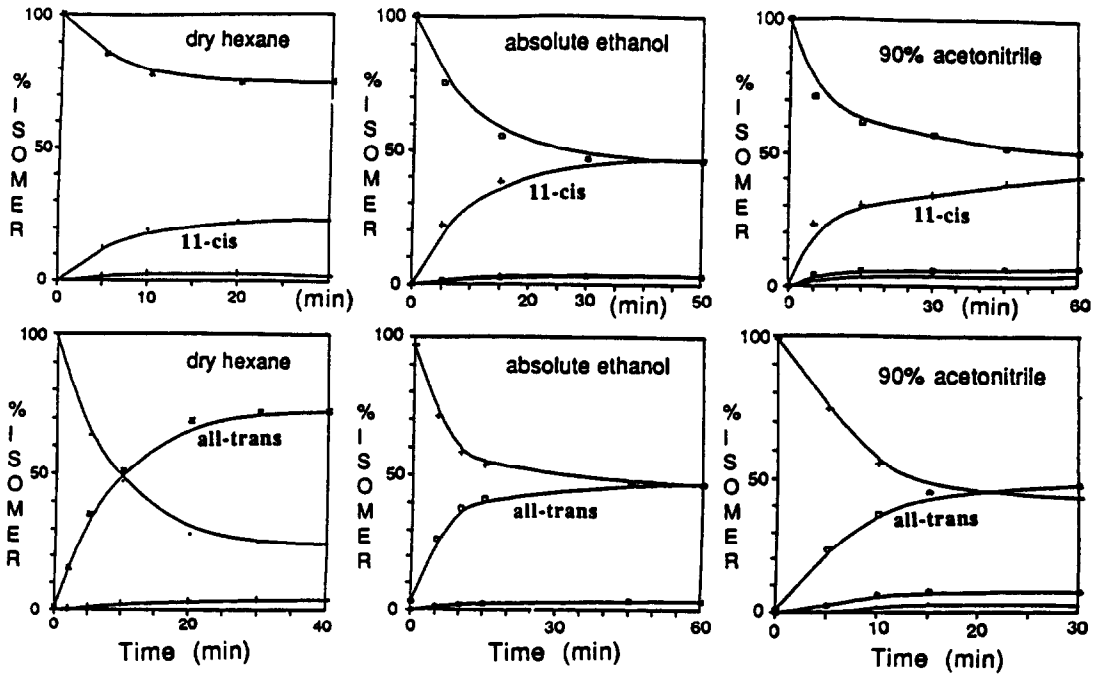


Figure 4. Progress of the isomerization reaction from direct irradiation of the all-trans (upper) and 11-cis isomers of the retinal analogs 2 in different solvents. The two minor product isomers are 9-cis and 13-cis (Table 1).

Table 1. Photostationary state compositions of retinal and analogs 1 and 2[a]

Compound	Solvent	% isomer at photostationary state[b]			
		13-cis	11-cis	9-cis	all-trans
1[c]	Hexane, dry	63.1[d]	17.4	--	19.6
	abs, EtOH	45.8	19.7	--	34.6
2[e]	Hexane, dry	--	23.7	2.8	73.4
	abs EtOH	3.4	47.1	3.1	46.5
	CH_3CN, H_2O (9:1)	3.1	41.7	6.6	48.7
Retinal[f]	Hexane, dry	27.4	--	2.7	69.9
	abs, EtOH	23.6	18.5	6.5	51.3

a. Deoxygenated solution (1×10^{-3} M) irradiated with 360nm light (200W Hanovia medium pressure Hg lamp, Corning 0-52 and 7-60 filters). b. Accuracy, ± 0.6%. c. From the trans isomer only. d. Overlapping a small amount of the 11,13-dicis isomer as revealed by nmr. e. From all-trans and 11-cis isomers. f. Data of X. Y. Li (ref. 6b).

around the unsubstituted 11,12-double bond is enhanced upon irradiation in a protic solvent.[6] A similar solvent effect for selective formation of isomers around the unsubstituted 9,10-bond in either compound **1** or **2** was not observed. This seemingly altered behavior is consistent with the generalization that the solvent stabilized excited dipolar intermediate exhibits a preference for isomerization near the more centrally located 11,12 bond in spite of the fact that a lower regioselectivity is usually exhibited by excited polyenes in protic solvents.[5] The vanishing amount of the 13-cis isomer formed is consistent with that observed for 13-demethylretinal.[16]

Binding interaction with bovine opsin. Four isomers of **1** and **2** were tested for possible visual pigment analog formation with bovine opsin. The results are summarized in Table 2.

Table 2. Properties of pigment analogs derived from isomers of 1 and 2

Retinal Analog	Pigment Absorption, nm (yield)	CD, nm	PSB, nm	OS cm^{-1} [b]
11-cis-1	475 (26%)	478, 338	433	2,040
11-cis-11-methylretinal[a]	498 (12%)			
11-cis-2	- (0%)			
9-cis-2	459 (13%)	461, 320	438	1,050
9,13-dicis-2	459 (7%)			
9-cis-11-methylretinal[a]	479 (9%)			

a. Unpublished results of M. Denny and A. E. Asato. b. Opsin shift.

At the first sight, it was unexpected that the 9-cis isomer of **2** gave a pigment analog (albeit in low yield) while the 11-cis isomer did not. However, the result is consistent with altered conformation of the polyene. The relocated 11-methyl in **1** and **2** introduces steric crowding nearby and causing nonplanarity around the 10,11 bond in the 9-cis isomers to a same extent as around the 12,13 bond in 11-*cis*-retinal. In fact, the CD spectrum of 9-*cis*-**2** (Figure 5) displays prominent α and β bands as in rhodopsin.[17] The negative result of the 11-cis isomer is probably due to a combined

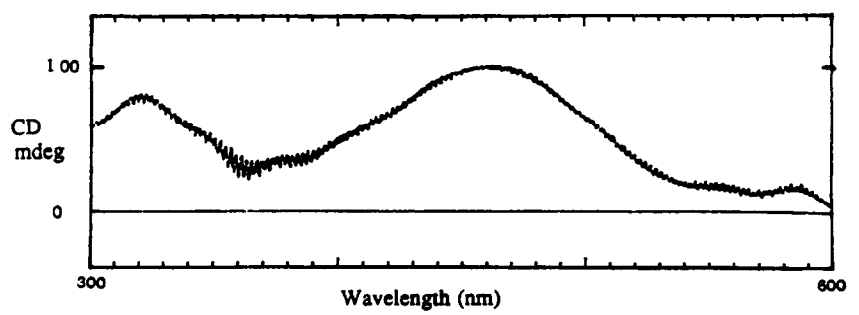


Figure 5. Circular dichroism spectrum of visual pigment analog of 9-*cis*-**2**.

effect of planarization of the chromophore and removal of the 9-methyl group which is known to interact tightly with the surrounding protein.[18] Thus, retaining the 13-methyl group as in **1** disallows planarization of the polyene chromophore. A pigment in a slightly higher yield than 9-*cis*-**2** results. This result also shows that the 11-methyl group probably does not overlap with nearby protein residues as suspected of the 12-methyl group in 12-methylrhodopsin.[4a] The lower pigment yield and smaller opsin shift value for 9-*cis*-**2** than those of 11-*cis*-**1** (Table 3) is consistent with the similar trend in 13-demethylrhodopsin.[19]

In summary, results in this paper reinforce the earlier conclusion that the location of methyl substituent has a significant effect on polyene photochemistry and conformation. The latter in turn has a noticeable effect on their spectroscopic properties and binding activity with opsin.

Experimental

Methods. Uv-vis spectra were recorded on a PE λ5 spectrometer, equipped with an Oxford C28009A cryo-tip cell for low temperature studies. Spectra at 78K were corrected for solvent (3MP) volume contraction (25%). ^{1}H-NMR spectra were obtained on a GE QE-300 spectrometer and to a limited extent an Omega GN-500 spectrometer. NOE studies were carried out on a Nicolet NT-300 spectrometer with degassed, sealed samples in CD_2Cl_2. CD spectra were obtained on a Jasco J-600 spectropolarimeter by taking the difference between those of the pigment and the bleached sample.

Photoisomerizations were carried out by irradiating nitrogen-saturated solutions (10^{-3} M) in Pyrex test tubes with filter 360nm light (Corning 0-52 and 7-60 filters) from a 200 W Hanovia lamp at 27°C. The course of isomerization was followed by hplc using an Altex ultrasphere 5-μ silica gel column. For **1**, the aliquots were allowed to equilibrate in the dark for 4 h prior to hplc analysis.

The rate of interconversion between 13-cis and 11,13-dicis isomers of **1** was monitored at 22°C by hplc (2% ethyl ether in hexane) using analytical Rainin Microsorb silica gel column. Regeneration of pigments and the preparation of cattle opsin were performed following procedures described earlier.[20]

Materials. β-cyclocitrylideneacetaldehyde was prepared following the methods of Tempel and Huisman[21] from β-ionone and of Ramamurthy et al.[10] from β-cyclocitral.

C_{15}-Triene ketone. Reaction of β-cyclocitrylideneacetaldehyde (980 mg, 5.5 mmol), acetone (25 ml) and 10% NaOH (10 ml) gave the desired product (794 mg) in 66% yield. ^{1}H-NMR: ($CDCl_3$, 300 MHz) δ 1.05 (s, 12-CH_3 and 13-CH_3) 1.75 (s, 14-CH_3), 2.29 (s, 15-CH_3), 6.12 (d, 10-H), 6.24 (dd, 8-H), 6.64 (d, 7-H) and 7.20 (dd, 9-H) ppm; $J_{7,8}$ = 15.6 Hz, $J_{8,9}$ = 10.9 Hz and $J_{9,10}$ = 15.5 Hz.

C_{18}-Tetraene ketone. Reaction of LDA (237 mg of diisopropylamine, 2.34 mmol) and n-butyllithium (0.72 ml, 2.5 M) in THF (15 ml) with ethyl trimethylsilylacetate (288 mg, 1.8 mmol) and the above C_{15}-triene ketone (196 mg, 0.90 mmol) gave the C_{17}-tetraene ester (11E/11Z ratio ~ 3) in 62% yield. ^{1}H-NMR ($CDCl_3$, 300 MHz): **all-trans**, δ 1.04 (s, 14-CH_3 and 15-CH_3), 1.73 (s, 16-CH_3), 2.33 (s, 17-CH_3), 5.76 (s, 12-H), 6.18 (dd, 8-H), 6.22 (d, 10-H), 6.37 (d, 7-H), 6.68 (dd, 9-H) ppm; $J_{7,8}$ = 15.8 Hz, $J_{8,9}$ = 10.5 Hz; $J_{9,10}$ = 15.6 Hz. **11-cis**, δ 1.04 (s, 16-CH_3 and 17-CH_3), 1.73 (s, 18-CH_3), 1.89 (s, 19-CH_3), 5.64 (s, 12-H), 6.28 (dd, 8-H), 6.38 (d, 7-H), 6.67 (dd, 9-H), 7.72 (d, 10-H) ppm; $J_{7,8}$ = 15.6 Hz, $J_{8,9}$ = 9.9 Hz and $J_{9,10}$ = 15.5 Hz. The ester (160 mg, 0.56 mmol) was hydrolyzed by reaction with KOH (1 g, 18 mmol), methanol (10 ml) and water (0.8 ml), and subsequently with methyl lithium (1.0ml, 1.3 M), giving an isomeric mixture of the ketone in 23% yield.

11-Methyl-9-demethylretinal. Reaction of NaH (8.5 mg, 60% oil dispersion) and diethyl cyanomethylphosphonate (37.6 mg, 0.21 mmol) in THF folowed by the addition of the C_{18}-ketone mixture (32 mg, 0.125 mmol in THF) gave an isomeric mixture of 11-methyl-9-demethylretinonitrile in 97% yield. To a dry hexane solution of the nitrile (33.7 mg, 0.12 mmol) was added DIBAL (0.3 ml,

Table 3. ^{1}H-nmr ($CDCl_3$) and uv/vis (hexane) data of isomers of 1 and 2

	Chemical Shift (ppm)									
Compound	11Me	13Me	7H	8H	9H	10H	12H	13H	14H	15H
all-trans-1	2.06	2.31	6.31	6.17	6.51	6.26	6.00	--	5.99	10.06
11-cis-1	2.02	2.27	6.32	6.17	6.53	6.69	5.86	--	6.02	10.07
13-cis-1	1.87	2.04	6.28	6.15	6.47	6.30	6.11	--	5.95	9.56
11,13-c-1	2.02	2.04	6.26	6.08	6.46	6.38	5.91	--	5.97	9.57
all-trans-2	2.09	--	6.38	6.21	6.63	6.32	6.33	7.51	6.17	9.61
11-cis-2	2.08	--	6.41	6.27	6.60	6.85	6.21	7.55	6.11	9.61
9-cis-2	2.17	--	6.33	6.66	6.25	5.91	6.39	7.47	6.16	9.62
9,13-c-2	2.12	--	6.34	6.67	6.25	5.94	7.13	7.32	5.85	10.20

	Coupling Constants						UV/VIS
Compound	7,8	8,9	9,10	12,13	13,14	14,15	λmax,nm (ε)
all-trans-1	15.6	10.2	14.8	--	--	8.3	353 (40,800)
11-cis-1	15.6	10.0	15.3	--	--	8.2	350 (28,000)
13-cis-1	15.7	10.3	15.4	--	--	8.0	314 (27,600)
11,13-dicis-1	15.7	10.3	15.4	--	--	8.3	299
all-trans-2	15.7	10.3	15.2	11.9	14.9	8.1	361 (42,700)
11-cis-2	15.6	10.2	15.1	12.1	14.9	7.9	357 (29,200)
9-cis-2	15.5	11.7	11.7	11.7	14.9	8.0	363 (30,400)
9,13-dicis-2	15.5	11.7	11.7	12.8	10.9	8.1	362 (14,200)

1.0 M) at -78°C. After usual workup, the expected retinal was obtained in 66% yield. The isomers were separated by hplc (4% ether in hexane, order of retention time: 11-cis and 11,13-dicis, 11-cis and all-trans). ^{1}H NMR: see Table 3.

C_{17}-Tetraene aldehyde. Reaction of NaH (560 mg, 60% oil dispersion) with C_5-phosphonitrile (3.2 g, 14.7 mmol) followed by β-cyclocitrylideneacetaldehyde (1.21 g, 6.8 mmol in THF) gave the C_{17}-nitrile (11E/11Z ratio ~ 1.9) in 39% yield. Alternatively, reaction of a mixture of 18-crown-6 (3.5 g, 13.5 mmol) bis-trifluoroethyl 2-methyl-3-cyano-2-propenylphosphonate[23] (550 mg, 1.7 mmol in 50 ml THF) and $KN(TMS)_2$ (5 ml, 0.5 M) with a solution of β-cyclocitrylideneacetaldehyde (303 mg, 1.7 mmol in THF). Upon usual workup, the 9-cis enriched nitrile mixture was obtained in 50% yield. Reduction of the nitrile (640 mg, 2.66 mmol) with DIBAL (4 ml, 1 M) gave the isomeric aldehyde mixture in 89% yield. The all-trans isomer was isolated by column chromatography. ^{1}H-NMR: ($CDCl_3$, 300 MHz) all-trans isomer δ 1.05 (s, 14, 15-CH_3), 1.74 (s, 16-CH_3), 2.30 (s, 17-CH_3), 5.9 (d, 12-H), 6.2 (dd, 8-H), 6.3 (d, 10-H), 6.5 (d, 7-H), 6.8 (dd, 9-H), 10.1 (d, CHO) ppm; $J_{7,8}$ = 15.6 Hz, $J_{8,9}$ = 10.6 Hz, $J_{9,10}$ = 15.3 Hz, $J_{12,13}$ = 8.2 Hz.

11-Methyl-9,13-didemethylretinal. Reaction of the isomeric mixture of the C_{17}-tetraene aldehyde (560 mg, 2.3 mmol) with the anion generated from NaH (200 mg, 60% oil dispersion) and diethyl cyanomethylphosphonate (730 mg, 4.1 mmol) yielded the expected nitrile in 42%. Subsequent reduction with DIBAL (1.2 ml, 1.0 M) gave 87% yield of 11-methyl-9,13-didemethylretinal. Hplc separation, using 4% ether in hexane, gave sequentially the following isomers: 9,13-dicis, 11-cis, 9-cis and all-trans. ^{1}H-NMR: see Table 3.

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