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A NOVEL RHODOPSIN ANALOGUE POSSESSING THE CYCLOPENTATRIENYLIDENE STRUCTURE AS THE 11-CIS-LOCKED AND THE FULL PLANAR CHROMOPHORE

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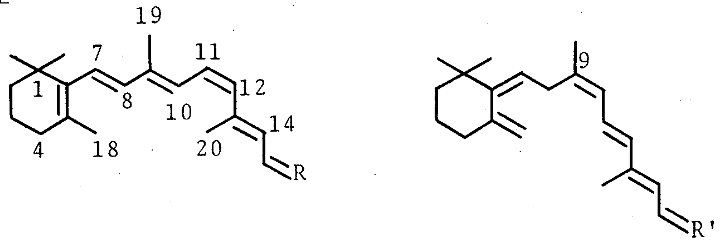
A short step synthesis of cyclopentatrienylidene retinals [(9)-(11)] is reported and the formation of the novel rhodopsin analogue (12) from the isomer (9) is described.

KEYWORDS —— rhodopsin analogue; 11-cis-retinal; retro-γ-retinal; primary photochemical event; cyclopentatrienylidene retinal; β-ionylidene-cyclopentenone

It is now commonly accepted that the chromophore of the visual pigment rhodopsin (1) is 11-cis-retinal (2) which binds in the form of a protonated Schiff base to the ε-amino group of a lysine in the apoprotein opsin. In our previous vision research,1) we have studied the chemistry of retro-γ-retinal as a new chromophore in rhodopsin analogues. From the photochemical behaviour at liquid nitrogen temperature with 9-cis-retro-γ-rhodopsin (3) prepared from 9-cis-retro-γ-retinal (4) and cattle opsin, we suggested that the conversion of rhodopsin to bathorhodopsin —— the primary photochemical event —— seems not to involve the proton translocation mechanism2) but to be caused by the isomerisation of the 11,12-cis-double bond to trans geometry. The side chain part of the chromophore in rhodopsin is assumed to be not the planar but the twisted 12-s-transoid conformer.3) However, the interaction between the chromophore 11-cis-retinal (2) and opsin in the rhodopsin molecule has not been solved in detail. As one of our extensive studies to clarify the interaction described above, we have been particularly interested in the properties of the artificial visual pigment (12) derived from the retinal analogue possessing the 11-cis-fixed and the full coplanar chromophore. We report here a short step synthesis of the cyclopentatrienylidene retinals [(9)-(11)] having the unique structure which the C-13-methyl group in 11-cis-retinal (2) binds to the C-10 position, and then the preparation of the novel visual pigment (12) derived from (9).

Reaction of the β-ionyl sulphone (5)4) with 4-acetoxy-cyclopentenone (6)5) using LDA (dry THF + HMPA, -30°C, under N_2, ca. 4 h) gave two β-ionylidenecyclopentenone isomers (7) and (8) [3 : 1, (ca. 24% yield)] which were clearly separated by low pressure liquid chromatography [Lobar column (LiChroprep Si 60, Merck) / n-hexane : benzene : ether = 10 : 10 : 1]. The less adsorbed isomer (7) [λ^{EtOH}_{max} 350 nm (ε17500); δ6) 6.30 (d,J=16,8-H), 8.14 (d,J=5.6,11-H), 3.05 (s,11'-H_2)] was assigned the <u>trans</u>-configuration7) on the newly formed double bond (C_9=C_{10}) in comparison with the ^{1}H-NMR data of the more strongly adsorbed isomer (8) [λ^{EtOH}_{max} 346 nm (ε14500); δ 6.66 (d,J=16,8-H), 8.22 (d,J=5.6,11-H), 3.03 (s,11'-H_2)] in which the proton signal at C-8 is deshielded by the 11,12-double bond.



(1) R=$\overset{+}{N}$H-opsin
(2) R=O
(3) R'=$\overset{+}{N}$H-opsin
(4) R'=O

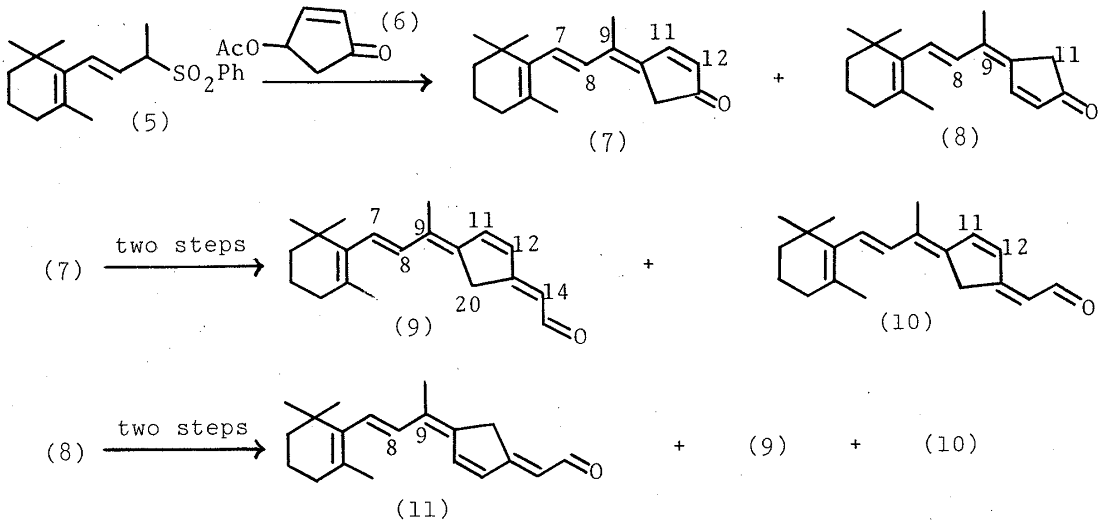
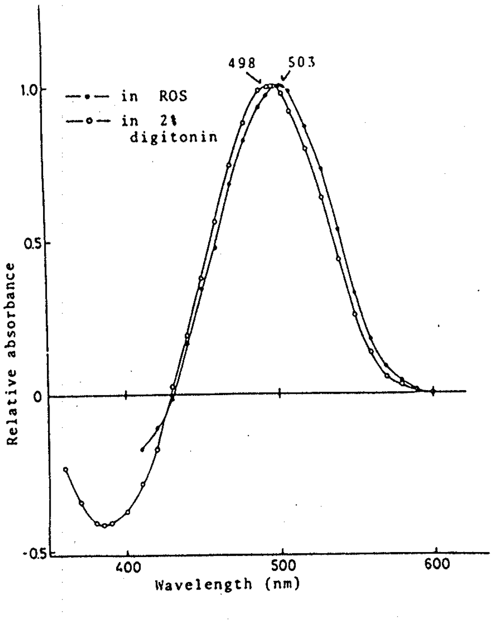


Table. Characteristic UV and ^{1}H-NMR data of the Cyclopentatrienylidene Retinals [(9)-(11)]

		11-<u>cis</u>-(9)	11,13-<u>dicis</u>-(10)	9,11-<u>dicis</u>-(11)
UV λ^{EtOH}_{max}		405 nm (ε18500)	392 nm (ε10000)	402 nm (ε11500)
^{1}H-NMR	7-H	6.32 (d,J=16)	6.33 (s-like)	6.27 (d,J=16)
δ_{ppm}	8-H	6.42 (d,J=16)		6.62 (d,J=16)
	11-H	7.27 (d,J=5)	7.36 (dd,J=5.5,1.5)	7.36 (d,J=5.5)
	12-H	6.61 (d,J=5)	7.28 (d,J=5.5)	6.59 (d,J=5.5)
	14-H	5.97 (td,J=7,1.5)	5.88 (dd,J=8,1.5)	5.98 (td,J=7,2)
	20-H_2	3.73 (d,J=1.5)	3.48 (s)	3.70 (d,J=2)

The proton signal at C-11 in the 9-cis-isomer (8) is also deshielded by the 7,8-double bond. An Emmons-Horner reaction of the 9-trans-isomer (7) with diethyl cyanomethylphosphonate (n-butyl-lithium, dry THF, 0°C, under Ar, 1 h) and subsequent reduction of the resulting nitrile with di-isobutylaluminium hydride (n-hexane, -70°C, under Ar, 10 min) led to a mixture (ca. 4 : 1) of the 11-monocis-aldehyde (9) and 11,13-dicis-isomer (10) in a low yield. Purification of each isomer was effectively accomplished by a combination of preparative TLC (SiO_2 / 15% ether in n-hexane) and HPLC (μ-Porasil (0.8 × 30 cm)/ n-hexane : benzene : ether = 16 : 16 : 1). In a similar procedure, the 9-cis-isomer (8) produced mainly the 9,11-dicis-aldehyde (11) accompanied by the isomers (9) and (10) which were carefully separated into the respective isomers by a preparative TLC followed by HPLC.[8] The structure of products [(9)-(11)] was confirmed on the basis of the ^{1}H-NMR peaks. Stereochemistry of the 13,14-double bond was determined from the chemical shifts of 20-H_2 signals which were strongly affected or not by the anisotropic effect of the aldehyde group. Geometry of the 9,10-double bond was also decided from the comparison with the chemical shifts of 8-H signals as in the case of (7) and (8). Characteristic UV and ^{1}H-NMR data of these isomers [(9)-(11)] are summarised in the Table. UV maximum values show that all three isomers have a high coplanarity in the conjugated part compared with those of other retinal isomers.[9] In addition, the protons at C-11 in the three isomers resonate at δca. 7.3 ppm which is very close to that of the all-trans-retinal having the most coplanar conjugated system in retinal analogues. The 11-cis-isomer (9) [λ_{max} 405 nm] was incubated with cattle opsin to yield the visual pigment (12) which showed an absorption maximum at 498 nm (Figure), the same value as rhodopsin.[10] This fact means that the chromophore in the rhodopsin molecule is not necessarily to be twisted at the 12-s-position. However, since the artificial pigment (12) derived from the aldehyde (9) was very unstable, the flexibility of the chromophoric part may be necessary for stable pigment formation. Studies on the other properties of the pigment (12) and the formation of other cyclopentatrienylidene rhodopsins are being pursued.

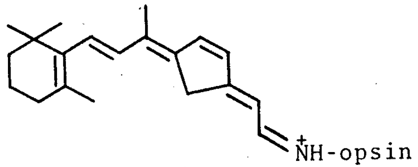


Figure

Difference Absorption Spectra of Visual Pigments formed from the 11-cis-Aldehyde (9) and Cattle Opsin

—●— : the pigment (λ_{max} 503 nm) in rod outer segments (ROS)

—○— : the pigment (λ_{max} 498 nm) in 2% digitonin



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6) ^{1}H-NMR spectra were determined on a 200 MHz superconducting NMR spectrometer using $CDCl_3$ solution.

7) Geometrical isomerism is expressed on the basis of the retinoidal polyene chain.

8) The fourth isomer, 9,11,13-tricis-aldehyde was detected by HPLC, but could not be isolated in a pure form because of its extreme instability.

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10) In a molar ratio, a 5-fold excess of (9) was incubated with cattle opsin [HEPES buffer (pH 7.0), 25°C, 48 h in the dark]. The pigment (12) was so labile in 20 mM NH_2OH that the difference absorption spectrum before and after heating it at 70°C for 1 min was calculated.

Recently, the artificial rhodopsins derived from the cycloheptatrienylidene retinals have been reported as nonbleachable rhodopsins retaining the full natural chromophore. H. Akita, S. P. Tanis, M. Adams, V. Balogh-Nair, and K. Nakanishi, J. Am. Chem. Soc., 102, 6370 (1980).

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