Communication

A Novel Rhodopsin Analog with Bicyclic Retinal Involving
the 8-18 Bonded Structure in the Chromophore

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(Received September 12, 1988)

Key Words bicyclic rhodopsin analog, CD spectrum of rhodopsin, bicyclic retinal, twisted conformation, fixed retinylidene chromophore, conjugated cyclopentadiene system

As part of our ongoing investigations to clarify the primary process of vision by chemical means, the elucidation of the origin of the induced CD spectrum (Table 1) of rhodopsin (**1**) (Scheme 1) is of particular interest for the conformational analysis of the photobleaching intermediates of rhodopsin, because CD spectrum gives precise information about the interaction between retinylidene chromophore and protein moiety of (**1**) in the intermediates (*I*). In the previous paper (2), it had been chemically confirmed from the CD data (Table 1) of the rhodopsin analog (**2**) involving the 10-20 bonded structure that the α -CD-band of (**1**) is due to the twisted 12s-*trans*-conformation in the chromophore. This is strong evidence supporting the theory for twisted chromophore (3-5) proposed for the induction of α -CD-band in (**1**). On the other hand, the rhodopsin analog (**2**) displayed a similar β -CD-band to that of (**1**), indicating that the β -CD-band is likely to be originated from a twisted conformation around the 6-7 single bond. This is also suggested in the case of the β -CD-band of 9-*cis*-rhodopsin (isorhodopsin) possessing 9-*cis*-retinal as its chromophore, because 9-*cis*-rhodopsin showed a similar β -CD-band (6) (Table 1) to that of (**1**) and the interaction between a β -ionone ring region of the chromophore and its binding site of opsin is similar in both pigments, (**1**) and isorhodopsin. Therefore, we synthesized the bicyclic rhodopsin analog-I (**3**) having the conformationally 6s-*cis*-fixed retinylidene chromophore and reported its CD data (7) (Table 1). Since the conjugated cyclopentadiene system in (**3**) was locked by the presence of a *gem*-

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Scheme 1

Table 1. CD data of rhodopsin (1) and its analogs.

Pigments	CD data (nm)	
	α -Band	β -Band
(1)	487 (+7.5) ^a	335 (+15.4)
(2)	negligible	336 (+11.6)
9- <i>cis</i> -Rhodopsin	484 (+8.9)	335 (+6.6)
(3)	527 (+16)	328 (−8.2)
(4)	512 (+13.6)	326 (−2.1)

^a Intensities in parentheses represent ellipticity (in mdeg/absorption).

dimethyl group at the 18-position, the bulkiness of which inversely caused the strong twist around the 8–9 single bond, resulting in the induction of negative β -CD-band in (3). We now report the synthesis and CD spectrum of a novel 9-*cis*-bicyclic rhodopsin analog-II (4) whose chromophore consists of an 8–18 bonded retinal analog (5) without a *gem*-dimethyl group in the cyclopentadiene system.

Aldol condensation (LDA, THF, $-78 \sim -40^\circ\text{C}$) of the bicyclic pentenone (6)(8) (Scheme 2) with the methyl ketone-acetal (7) gave the hydroxy-acetal (8) in 83% yield as a mixture (1:1) of diastereoisomers which, without separation, was deprotected to afford the hydroxy-aldehyde (9) in 80% yield. An Emmons-Horner reaction (*n*-BuLi, THF, 0°C) of (9) with the ester-phosphonate (10) gave the diene-ester (11) in 68% yield as a mixture of four isomers (13-*trans*- and -*cis*-isomers for each of the two diastereoisomers) which, without separation, after reduction of the ketone group with LiBH₄ was dehydrated (I₂, pet. ether, reflux, 1 h) to provide the conjugated pentaene-ester (12) in 20% yield. Conversion of the ester group in (12) to the aldehyde group led to a mixture of conjugated pentaene-aldehydes in 52% yield, the repeated purification of which by a combination of column chromatography and preparative HPLC in the dark furnished four bicyclic retinal isomers

Table 2. Characteristic UV-VIS and ^1H -NMR data for bicyclic retinals (**13**, **15**, and **17**).

		Bicyclic retinal-II		Bicyclic retinal-I
		all- <i>trans</i> (13)	all- <i>trans</i> (15)	all- <i>trans</i> (17)
UV-VIS $\lambda_{\text{max}}^{\text{EtOH}}$ nm		425 (ϵ 32,000)	425 (ϵ 29,000)	425 (ϵ 33,100)
		295 (ϵ 10,000)	295 (ϵ 11,000)	292 (ϵ 8,300)
^1H -NMR (200 MHz) (δ , CDCl_3)	9-Me	2.07 (s)	2.09 (s)	2.11 (s)
	13-Me	2.33 (s)	2.33 (s)	2.34 (s)
	18-H ₂	3.16 (brs)	3.12 (sharp s)	—
	12-H	6.39 (d, $J=16$)	6.36 (d, $J=15$)	6.42 (d, $J=15$)
	11-H	7.15 (dd, $J=16, 11.5$)	7.15 (dd, $J=15, 11.5$)	7.21 (dd, $J=15, 11$)
	10-H	6.40 (d, $J=11.5$)	6.36 (d, $J=11.5$)	6.43 (d, $J=11$)
	7-H	6.45 (s)	6.63 (s)	6.47 (s)
	CHO	10.10 (d, $J=8.5$)	10.10 (d, $J=8.5$)	10.10 (d, $J=8$)

Scheme 3 NOE Data of (**13**) and (**15**)

Scheme 4

[(**13**):(**14**):(**15**):(**16**)=1.2:1.0:1.7:1.4]. The structures of the isomers were determined on the basis of the UV-VIS and ^1H -NMR spectral data (Table 2) compared with those (**7**) of another all-*trans*-bicyclic retinal (**17**) having a fixed conjugated pentaenal chromophore. Two bicyclic retinals (**13** and **15**) had absorption maximum values (425 nm) identical with that of (**17**), showing that both possess the same chromophoric system as (**17**). Confirmation of their stereostructures was based on measurements of nuclear Overhauser effects (NOE) (Scheme 3). Irradiation of the two all-*trans*-bicyclic retinals (**13** and **15**) using a fluorescent lamp (30 W) in MeOH produced 9-*cis*-isomers (**5** and **18**) respectively (Scheme 4) in *ca.* 70% yield. The 9-*cis*-geometry for (**5**) and (**18**) was recognizable from the upfield

shift of the 10-H signal and the downfield shift of the 11-H signal in their $^1\text{H-NMR}$ by comparison with those of all-*trans*-isomers (**13** and **15**) (**9**). Their UV-VIS absorption maxima (417 nm) and $^1\text{H-NMR}$ data** indicate that (**5**) and (**18**) have a coplanar structure in the 6–10 region of the molecule.

In a binding experiment [2% digitonin, 10 mmol HEPES buffer (pH 7.0), 25°C in the dark, 48 h] using bovine opsin, the 9-*cis*-isomer (**5**) with a *gem*-dimethyl group at the 4-position gave a new bicyclic rhodopsin analog (**4**), whereas the other 9-*cis*-isomer (**18**) with a *gem*-dimethyl group at the 1-position produced a negligible amount of artificial pigment***. The novel rhodopsin analog (**4**) showed an absorption maximum at 519 nm and an interesting CD spectrum (Table 1). A very weak β -CD-band in (**4**) suggests that the β -band in the induced CD of rhodopsin originates from the twist of a double bond system next to the β -ionone ring moiety. It was reported that rhodopsin analogs derived from 5,6-dihydroretinal (**6**) and 5,6-epoxy-3-dehydroretinal (**10**) having saturated rings showed no β -CD-band, and retro- γ -rhodopsins (**11**) possessing two dissected chromophoric system composed of a planar ring exhibited the very weak β -CD-band. These results also support the conclusion that the β -CD-band of rhodopsin originates from the twist of the 6–7 single bond.

We gratefully thank Professor K. Nakanishi, Columbia University in the City of New York, for his valuable discussion.

This work was supported in part by a Grant-in-Aid (60304098) for Cooperative Research from the Ministry of Education, Science and Culture of Japan, to M.I. and T.Y.

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** Characteristic $^1\text{H-NMR}$ data for (**5**) and (**18**): δ (200 MHz, CDCl_3), (**5**): 2.06 (3H, s, 9-Me), 2.32 (3H, s, 13-Me), 3.20 (2H, brs, 18- H_2), 6.32 (1H, d, $J=16$ Hz, 12-H), 6.07 (1H, d, $J=12$ Hz, 10-H), 6.45 (1H, s, 7-H), 7.37 (1H, dd, $J=16, 12$ Hz, 11-H), 10.10 (1H, d, $J=8$ Hz, CHO). (**18**): 2.07 (3H, s, 9-Me), 2.30 (3H, s, 13-Me), 3.17 (2H, sharp s, 18- H_2), 6.32 (1H, d, $J=16$ Hz, 12-H), 6.06 (1H, d, $J=12$ Hz, 10-H), 6.63 (1H, s, 7-H), 7.36 (1H, dd, $J=16, 12$ Hz, 11-H), 10.10 (1H, d, $J=8.5$ Hz, CHO).

*** The result that (**18**) failed to bind with opsin was unexpected. The interaction between the analogs having the cyclopentadiene structure and the β -ionone ring binding site of opsin is now under investigation.

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