A Novel Rhodopsin Analogue possessing the Conformationally 6-s-*cis*-Fixed Retinylidene Chromophore

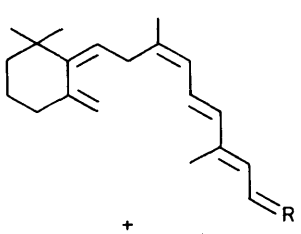
Masayoshi Ito,*[a] Tomomi Hiroshima,[a] Kiyoshi Tsukida,[a] Yoshinori Shichida,[b] and Tōru Yoshizawa[b]

[a] *Kobe Women's College of Pharmacy, Motoyamakita-machi, Higashinada-ku, Kobe 658, Japan*
[b] *Department of Biophysics, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606, Japan*

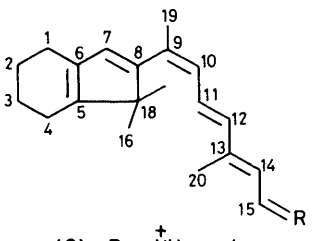
The conformationally 6-s-*cis*-fixed rhodopsin analogue (**3**), which shows interesting spectroscopic properties, has been synthesised.

In the course of our studies to clarify the primary process of vision by chemical methods, two types of rhodopsin analogue [(**1**)[1] and (**2**)[2]] have been synthesised. The former possesses two separated chromophore systems, while the latter has an 11-*cis*-locked cyclopentenediylidene chromophore. Photochemical studies[3,4] of these analogues showed that an 11-*cis* to 11-*trans* isomerisation is a prerequisite for visual transduction. The artificial pigment (**2**) having a non-twisted conformation around the 12-s-*trans* bond showed an interesting c.d. spectrum[4] [α-band: negligible, β-band: 336 nm (+11.6)†]. Comparison of the c.d. data for (**2**), rhodopsin[5] [α-band: 487 nm (+7.5), β-band: 335 nm (+15.4)], and cycloheptenediylidene rhodopsin[5] [α-band: 488 nm (+14.3), β-band: 330 nm (+16.9)] demonstrated that the α-c.d. band of rhodopsin has its origin in the twisted 12-s-*trans*-conformation in the chromophore. According to the theory for twisted chromophores[6] proposed for the induction of c.d. in rhodopsin, the β-band is assumed to originate from ring–side chain interaction in the chromophore. A conformationally 6-s-*cis*-fixed rhodopsin analogue would thus be of particular interest in investigating this subject. We now report the preparation and spectroscopic properties of the 9-*cis*-bicyclic rhodopsin analogue (**3**) whose chromophore consists of the novel 6-s-*cis*-locked retinal analogue (**5**).

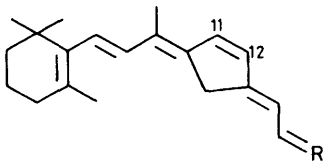
Aldol condensation (1 M-NaOH) of the 9-*trans*-aldehyde (**6**)[7] with excess of acetone gave the all-*trans*-ketone (**7**) in 80% yield, which was transformed by Emmons–Horner reaction with cyanomethyl phosphonate (Bu^nLi) into the pentaenenitrile (**8**) in 76% yield. Reduction of the nitrile group (Bu^i_2AlH in n-hexane) followed by purification (preparative t.l.c. and h.p.l.c.) afforded the bicyclic retinal (**9**)‡ in



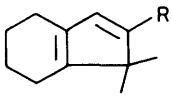
(**1**) R = $\overset{+}{N}H$–opsin

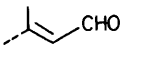


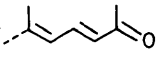
(**3**) R = $\overset{+}{N}H$–opsin
(**4**) R = $\overset{+}{N}H$–Bu^n
(**5**) R = O

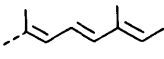


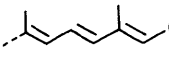
(**2**) R = $\overset{+}{N}H$–opsin

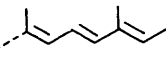


(**6**) R = 

(**7**) R = 

(**8**) R = 

(**9**) R = 

(**10**) R = 

(**11**) is 9-*cis*, 13-*cis*-(**9**).

† Intensities in parentheses represent ellipticity (in mdeg/absorption).

‡ Alternatively, this aldehyde was derived from the corresponding ester (**10**)[7] in low yield (10%) by reduction ($LiAlH_4$) and oxidation (MnO_2).

54% yield. Spectral data indicated that the aldehyde (**9**) had the all-*trans* stereochemistry [λ(EtOH): 425 (ε 33 100) and 292 (ε 8 300) nm; δ_H ($CDCl_3$): 1.18 (6H, s, *gem*-Me_2), 2.11 (3H, s, 9-Me), 2.34 (3H, s, 13-Me), 6.42 (1H, d, *J* 15 Hz, 12-H), 6.43 (1H, d, *J* 11 Hz, 10-H), 6.47 (1H, s, 7-H), 7.21 (1H, dd, *J* 15 and 11 Hz, 11-H), and 10.10 (1H, d, *J* 8 Hz, 15-H); δ_C ($CDCl_3$): 15.6 (19-C) and 13.1 (20-C). The electronic absorption maximum of (**9**) is at a much longer wavelength than that (380 nm) of all-*trans*-retinal, showing that the bicyclic retinal (**9**) has a highly coplanar conjugated system. Irradiation of the all-*trans*-aldehyde (**9**) using a high pressure (300 W) Hg lamp with a Pyrex filter in MeOH produced an isomeric mixture (all-*trans*:9-*cis*:9-*cis*,13-*cis* 7:20:3), preparative t.l.c. and h.p.l.c. of which gave the new 9-*cis*- (**5**) and 9-*cis*,13-*cis*- (**11**) isomers in pure form, characterised spectroscopically [(**5**), λ(EtOH): 370 (ε 13 100) and 335 (ε 14 800) nm; δ_H($CDCl_3$): 1.07 (6H, s, *gem*-Me_2), 2.07 (3H, s, 9-Me), 2.20 (3H, s, 13-Me), 5.99 (1H, s, 7-H), 6.20 (1H, d, *J* 11 Hz, 10-H), 6.26 (1H, d, *J* 15.5 Hz, 12-H), 6.92 (1H, dd, *J* 15.5 and 11 Hz, 11-H), and 10.05 (1H, d, *J* 8 Hz, 15-H); δ_C($CDCl_3$): 22.4 (19-C) and 13.4 (20-C)]. The *cis*-geometry of the 9,10-double bond in (**5**) was mainly deduced from the ^{13}C n.m.r. isomerisation shift[8] for 19-C. The upfield shift of the ^1H resonances in (**5**) relative to those of (**9**), and the hypsochromic shift of the u.v. absorption maximum in (**5**) suggest that single bonds in its chromophore are strongly twisted.

The 9-*cis*-isomer (**5**) bound with cattle opsin [2% digitonin, 10 mmol HEPES buffer (pH 6.86)] to give the novel 9-*cis*-rhodopsin analogue (**3**) which exhibited an absorption maximum at 539 nm, the longest wavelength observed so far for visual pigment analogues with the chromophore possessing the same number of conjugated double bonds as that of 9-*cis*-rhodopsin. Although the absorption maxima of both pigments differ considerably, the difference (2590 cm^{-1}) in wavenumber between the absorption maxima of the protonated Schiff base (SBH^+) (**4**) (473 nm) and the pigment (**3**) is close to that (2110 cm^{-1})[9] for 9-*cis*-retinal-SBH^+ and 9-*cis*-rhodopsin. This indicates that in the artificial pigment (**3**), there are similar interactions with the binding site of the protein as in the case of 9-*cis*-rhodopsin. Thus, the pigment (**3**) is the first 6-s-*cis*-locked[10] example of a visual pigment analogue involving the same chromophore as 9-*cis*-rhodopsin. In addition, (**3**) showed an interesting c.d. spectrum [α-band: 527 nm (+16), β-band: 328 nm (−8.2)]. A negative sign for the β-c.d.-band has not been reported so far for artificially prepared visual pigments. It is likely that a chirally twisted chromophore having a partially constrained planar structure plays an important role in the c.d. spectrum. The present results should be promising for conformational analysis of the photobleaching intermediates of rhodopsin whose spectra (absorption and c.d.) have been reported[11] to change markedly under different conditions.

We thank Miss N. Amesaka and Miss M. Muro for technical assistance.

Received, 23rd April 1985; Com. 538

References

1 M. Ito, K. Hirata, A. Kodama, K. Tsukida, H. Matsumoto, K. Horiuchi, and T. Yoshizawa, *Chem. Pharm. Bull.*, 1978, **26**, 925.
2 M. Ito, A. Kodama, K. Tsukida, Y. Fukada, Y. Shichida, and T. Yoshizawa, *Chem. Pharm. Bull.*, 1982, **30**, 1913.
3 S. Kawamura, T. Yoshizawa, K. Horiuchi, M. Ito, A. Kodama, and K. Tsukida, *Biochim. Biophys. Acta*, 1979, **548**, 147.
4 Y. Fukada, Y. Shichida, T. Yoshizawa, M. Ito, A. Kodama, and K. Tsukida, *Biochemistry*, 1984, **23**, 5826.
5 H. Akita, S. P. Tanis, M. Adams, V. Balogh-Nair, and K. Nakanishi, *J. Am. Chem. Soc.*, 1980, **102**, 6370.
6 W. Sperling and C. N. Rafferty, *Nature (London)*, 1969, **224**, 591; M. J. Burke, D. C. Pratt, R. R. Faulker, and A. Moscowitz, *Exp. Eye Res.*, 1973, **17**, 557; T. G. Ebrey and T. Yoshizawa, *ibid.*, 1973, **17**, 543.
7 A. Kodama, M. Ito, and K. Tsukida, *Chem. Pharm. Bull.*, 1982, **30**, 4205.
8 G. Englert, *Helv. Chim. Acta*, 1975, **58**, 2367; L. Carey, J. M. Clough, and G. Pattenden, *J. Chem. Soc., Perkin Trans. 1*, 1983, 3005.
9 M. G. Motto, M. Sheves, K. Tsujimoto, V. Balogh-Nair, and K. Nakanishi, *J. Am. Chem. Soc.*, 1980, **102**, 7947.
10 An artificial pigment derived from the naphthyl analogue of retinal and bacterio-opsin has been reported (M. Akhtar, M. L. Jallo, and A. H. Johnson, *J. Chem. Soc., Chem. Commun.*, 1982, 44) but the pigment does not possess the same chromophore as rhodopsin.
11 T. Yoshizawa and Y. Shichida, *Methods Enzymol.*, 1982, **81**, 634.