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DETERMINATION OF 6s-*TRANS* CONFORMATION OF RETINAL CHROMOPHORE IN SENSORY RHODOPSIN I AND PHOBORHODOPSIN

Akimori Wada,[a] Akiko Akai,[a] Takehiko Goshima,[b] Tetsuo Takahashi,[b] and Masayoshi Ito[a*]

[a] *Kobe Pharmaceutical University, 4-19-1, Motoyamakita-machi, Higashinada, Kobe 658-8558, Japan*

[b] *Japan Advanced Institute of Science and Technology, Hokuriku, 15, Asahidai , Tatsunokuchi, Ishikawa 923-1211, Japan*

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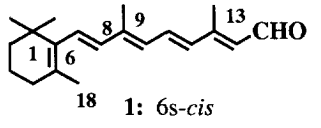
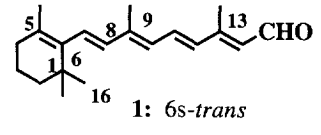
Abstract: 8,16-Ethanoretinal **3** was synthesized from 2-methylcyclohexanone. From the binding experiments of **3** and 8,18-ethanoretinal **2** with apoproteins of sensory rhodopsin I and phoborhodopsin, it was found that retinal was incorporated as 6s-*trans* conformation in the both proteins.

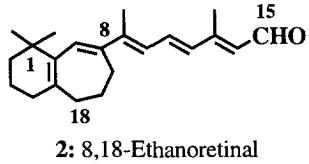
It is well known that a retinal molecule **1** is a chromophore of protein pigments such as rhodopsin, bacteriorhodopsin, retinochrome and so on.[1] These proteins exhibit the significant functions in vital cells respectively, and the conformation of retinal chromophore in the proteins plays an important role. In past two decades, a number of reports have appeared on dealing with the synthesis of retinal analogs for examining the structure and protein environment of the retinal cromophore in the pigments.[2] In order to investigate the conformation around the cyclohexene ring of chromophore (6s-*cis* or 6s-*trans* conformation) in sensory rhodopsin I and phoborhodopsin, we describe here the synthesis of (all-*E*)-8,16-ethanoretinal **3**, in which C8 and C16 positions in **1** are connected by an ethylene group, and the binding experiments of **3** and (all-*E*)-8,18-ethanoretinal **2**[3] having the 6s-*cis* conformation with apoproteins.



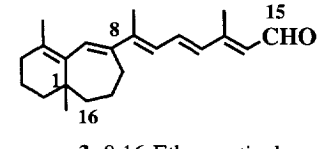
1: 6s-*cis*



1: 6s-*trans*



2: 8,18-Ethanoretinal



3: 8,16-Ethanoretinal

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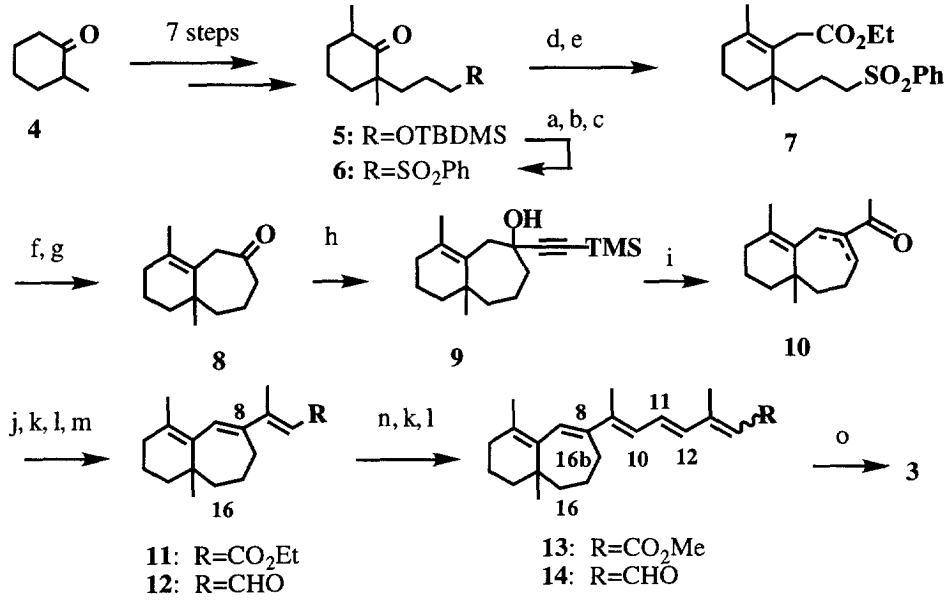
PII: S0960-894X(98)00233-9

The silyloxy ketone **5**, derived from 2-methylcyclohexanone by the previously reported method[4] with a little modification, was converted to the sulfonyl ketone **6** by the sequence of deprotection, bromination and sulfonylation. Treatment of **6** with the lithium salt of ethyl acetate followed by dehydration using thionyl chloride afforded the sulfone ester **7** as a sole product. Cyclization of **7** using lithium bis(trimethylsilyl)amide (LiHMDS) and subsequent desulfonylation employing tributyltin hydride[5] gave the bicyclic ketone **8**.[6,7] Trimethylsilylethynylation of **8** with trimethylsilylethynylcerium (III) reagent,[8] prepared from trimethylsilylethynyllithium and cerium (III) chloride in THF, afforded the alcohol **9** in high yield. Treatment of the adduct **9** with formic acid[9] caused dehydration, desilylation, hydration and keto-enol isomerization at the same time to give the methyl ketone **10** as an isomeric mixture of double bond (3:2). The condensation of **10** with triethyl phosphonoacetate using NaH provided the ester **11** as a single isomer. The conversion of **11** to the aldehyde **12**[6,10] was achieved by $LiAlH_4$ reduction, MnO_2 oxidation and subsequent HPLC separation. The geometry of the 9, 10 double bond was decided as (*E*) from the strong NOESY correlation between 10-H and 16b-H in its NMR spectra. The Emmons-Horner reaction of **12** with C5-phosphonate was carried out using *n*-BuLi to give the ester **13**, in which the



a) TBAF / THF, r.t., b) PBr_3, r.t., c) $PhSO_2Na$, TBAI / C_6H_6-H_2O-acetone, reflux; 92% from **5**, d) LDA, AcOEt / THF, -78°C; 82%, e) $SOCl_2$ / pyridine, 0°C; 82%, f) LiHMDS / THF, -78°C, g) Bu_3SnH, toluene; 82% from **7**, h) TMS-C≡C-$CeCl_2$ / THF, -78°C; 85%, i) 85% HCO_2H, reflux; 78%, j) NaH, $(EtO)_2P(O)CH_2CO_2Et$ / 10% DMF-THF, reflux; 75%, k) $LiAlH_4$ / Et_2O, 0°C, l) MnO_2 / CH_2Cl_2, r.t., m) HPLC; 13% from **10**, n) *n*-BuLi, $(EtO)_2P(O)CH_2(CH_3)C{=}CHCO_2Me$ / THF, -78°-0°C; 83%, o) column chromato.; 54%.

geometry of the 11,12 double bond was determined as (*E*) from the coupling constant of 11-H signal in its NMR. The final transformation of **13** to the corresponding aldehyde **14** was established according to the usual method by $LiAlH_4$ reduction and MnO_2 oxidation and the (all-*E*) isomer **3**[6, 10] was isolated in pure form by flash column chromatography.

Subsequently, the binding experiments of **2** and **3** with apoproteins in membrane preparations from *Halobacterium salinarum* (formerly *halobium* or *salinarium*) were carried out according to the previously reported methods[11] and the protonated Schiff bases (PSB) of **2** and **3** with *n*-butylamine were formed by the usual method. The absorption maxima and opsin shifts of artificial and natural pigments are shown in the Table. In sensory rhodopsin I, for the first time, the conformation around the 6-7 single bond of retinal chromophore is chemically substantiated using 6s-*trans* fixed retinal analog **3** and it was strongly suggested that in phoborhodopsin the retinal is incorporated as 6s-*trans* conformation on the basis of opsin shift values of pigments. However, apoprotein of phoborhodopsin has a loose cavity of chromophore binding site compared to that of sensory rhodopsin I. Further investigation is currently in progress.

Table. Absorption Maxima and Opsin Shifts of Pigments.

Chromophores	Aldehydes[a] λ max / nm	PSB[b] λ max / nm	sRh[c] λ max / nm	Opsin Shifts Δ ν / cm^{-1}	pRh[d] λ max / nm	Opsin Shifts Δ ν / cm^{-1}
(all-*E*)-Retinal (**1**)	381	443	587	5500	487	2000
(all-*E*)-8,18-Ethanoretinal (**2**)	382	449	–e	– e	464	670
(all-*E*)-8,16-Ethanoretinal (**3**)	373	437	563	5100	466	1400

a) In ethanol. b) In methanol. c) Sensory rhodopsin I. d) Phoborhodopsin.
e) No pigment formed.

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6. Satisfactory ^{1}H-NMR, IR and MS spectral data were obtained.

7. ^{1}H-NMR data for compound **8** is as follows; (300 MHz, $CDCl_3$) δ 1.07 (3H, s, Me), 1.2-1.4 (2H, m, one of $CH_2 \times 2$), 1.5-1.8 (1H, m, one of CH_2), 1.63 (3H, s, 1-Me), 1.9-2.1 (5H, m, $CH_2 \times 2$, one of CH_2), 2.1-2.3 (2H, m, CH_2), 2.4-2.5 (2H, m, CH_2), 3.11 (1H, d, *J*=14, one of $COCH_2$), 3.17 (1H, d, *J*=14, one of $COCH_2$).

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10. ^{1}H-NMR data for compounds **12** and **3** are as follows:
For **12**: (300 MHz, $CDCl_3$) δ 0.97 (3H, s, Me), 1.2-1.9 (8H, m, $CH_2 \times 4$), 1.65 (3H, s, Me), 1.9-2.1 (2H, m, CH_2), 2.2-2.4 (2H, m CH_2), 2.35 (3H, s, Me), 6.10 (1H, d, *J*=8, CH), 6.74 (1H, s, CH), 10.13 (1H, d, *J*=8, CHO); For **3** : (300 MHz, $CDCl_3$) δ 0.97 (3H, s, Me), 1.30-1.42 (2H, m, one of $CH_2 \times 2$), 1.46-1.52 (1H, m, one of CH_2), 1.60-1.76 (5H, m, $CH_2 \times 2$, one of CH_2), 1.64 (3H, s, Me), 1.98-2.04 (2H, m, CH_2), 2.07 (3H, s, Me), 2.32-2.42 (2H, m, CH_2), 2.33 (3H, s, Me), 5.80 (1H, d, *J*=8, CH), 6.35 (1H,d, *J*=11, CH), 6.37 (1H, s, CH), 6.44 (1H, d, *J*=15, CH), 7.15 (1H, dd, *J*=15, 11, CH), 10.11 (1H, d, *J*=8, CHO).

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