

Synthesis of 8,16-Ethanoretinals and Their Interactions with Apoprotein of Phoborhodopsin from *Natronobacterium pharaonis*

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Abstract: Several 8,16-Ethanoretinals **3** were synthesized from cyclohexanones. From the binding experiments of these analogs with apoprotein of phoborhodopsin, it was found that retinal was incorporated as *6s-trans* conformation in the protein and the fine structure in absorption spectra of pigments were dependent on the coplanarity of the conjugated polyene structure in the chromophore.

Keywords: 8,16-Ethanoretinal, retinal analog, phoborhodopsin, chromophore, *s-trans* conformation.

INTRODUCTION

It is well known that the retinal molecule **1** is a chromophore of photoreceptor proteins, and binds to Lys

are phototaxis receptors transducing attractant and repellent migratory responses, respectively [3]. Among these pigments, sRII has two characteristic features in its absorption spectrum. The first is that sRII absorbs

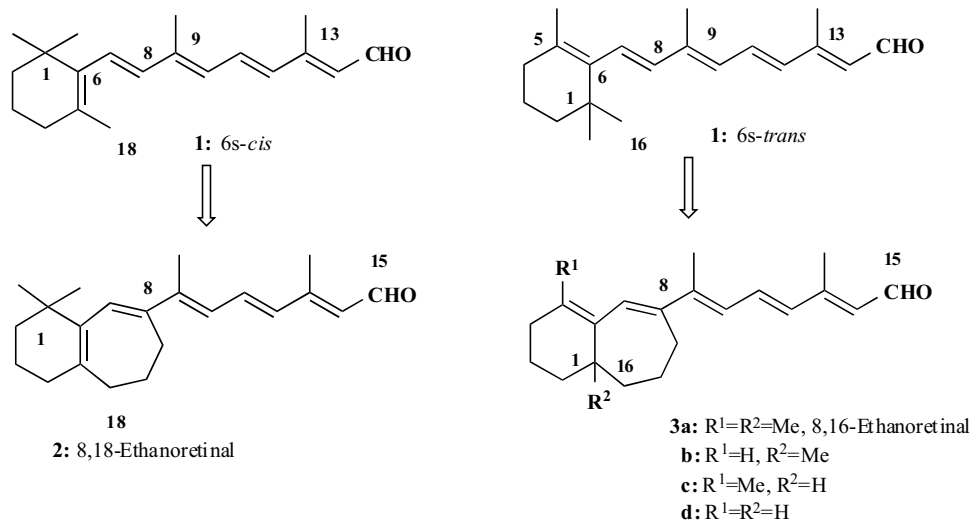


Fig. (1). Chemical structure of retinal and its analogs.

residue in helix G of the seven transmembrane α -helix of proteins [1]. There are four kinds of pigments in haloarchaea, such as *Halobacterium salinarum* and *Natronobacterium pharaonis*, and they have two distinctly different functions by common photochemical reactions: light-driven ion transport and photosensory signaling. Bacteriorhodopsin (bR) and halorhodopsin (hR) are light driven ion pumps for protons and chloride ions [2], respectively, and the sensory rhodopsins I and II (sRI and sRII or phoborhodopsin = pR)

maximally near 500 nm, almost the same λ max of the visual pigment rhodopsin, and this is about 60 nm shorter than those of another pigments [3]. The second point is the fine structure in its spectrum, which confers a shoulder at 460 nm in sRII spectrum in contrast to simple bell-shaped spectra of other retinylidene proteins. For their biological function in respective vital cells, the conformation of the chromophore retinal in the proteins plays an important role. In the 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 chromophore in the pigments [4]. In order to investigate the conformation around the cyclohexene ring of chromophore (*6s-cis* or *6s-trans* conformation) in sRI and sRII, we have synthesized (all-*E*)-8,16-ethanoretinal **3a** (6s-

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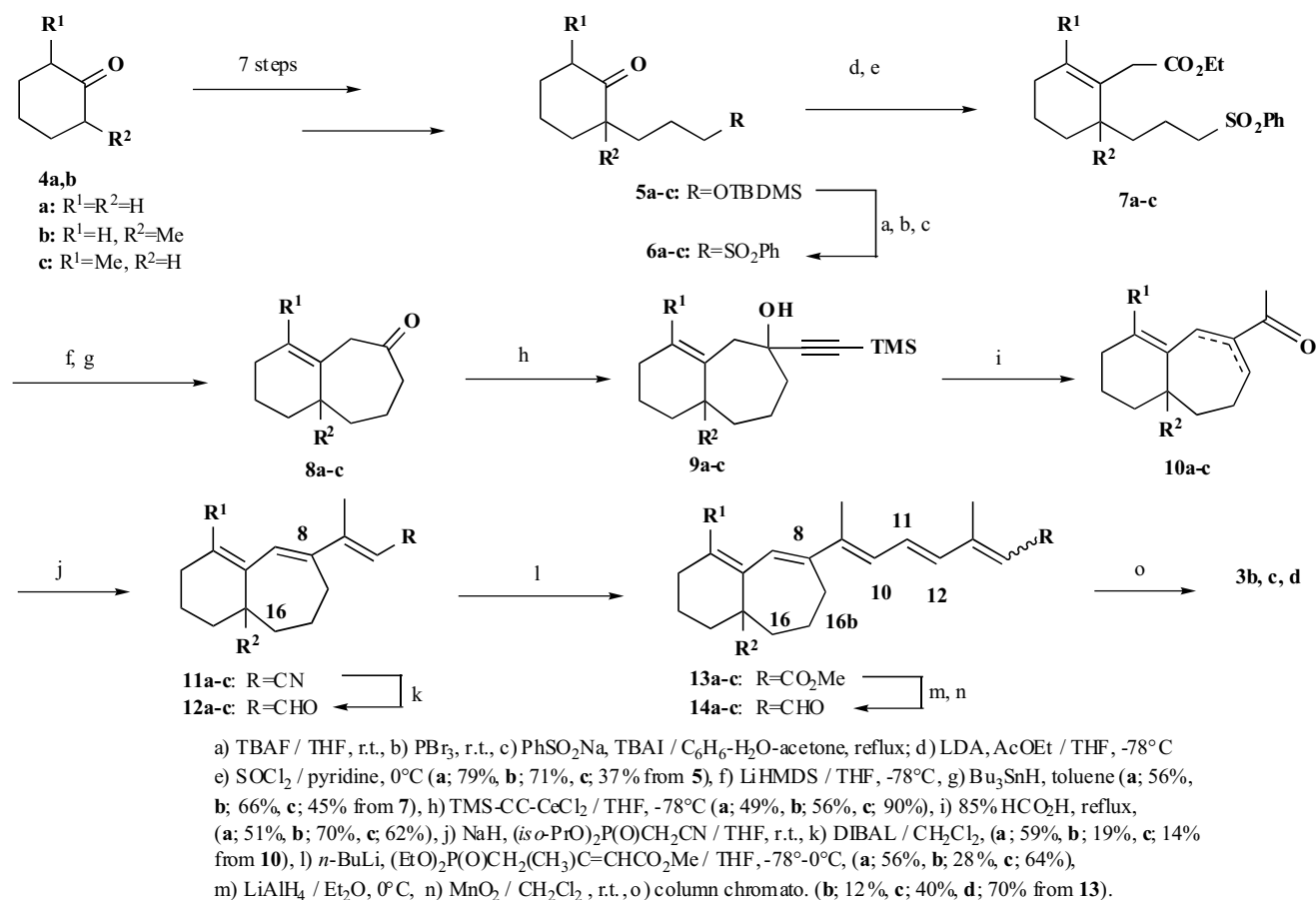


Fig. (2). Synthetic pathway for demethylated 8,16-ethanoretinals.

trans conformation) and (all-*E*)-8,18-ethanoretinol **2** (6*s-cis* conformation) (Fig. 1) [5, 6]. From the binding experiments of these analogs with apoproteins, it was strongly supported that retinal was incorporated as 6*s-trans* conformation in the protein [5]. In connection with our study, especially to elucidate the origin of the fine structure in the absorption spectrum of sRII, we describe here the synthesis of demethylated (all-*E*)-8,16-ethanoretinols **3b-d**, and the binding experiments of **3** with apoprotein.

CHEMISTRY

The silyloxy ketones **5a,b** were derived from cyclohexanone and 2-methylcyclohexanone by the previously reported method [7] with a little modification. The ketone **5c** was prepared from **5a** by the reaction of methyl iodide using lithium diisopropylamide (LDA) as a base. These ketones **5** were converted to the bicyclic ketones **8** via the corresponding sulfonyl esters **7**, and then transformed into the retinal analogs **3** in the same manner as described for the synthesis of **3a** (Fig. 2)[§]. The structure of **3b-c** was

determined as all-*E* from the comparison of their ¹H-NMR spectra with those of **3a** and retinal isomers [5, 6].

BINDING EXPERIMENT

In the binding experiments of **2** and **3**, the apoprotein in membrane preparations from *Natronobacterium pharaonis* (ppR) was used and binding procedure was conducted according to the previously reported methods [8]. The protonated Schiff bases (PSB) of **2** and **3** with *n*-butylamine were formed by the usual method. The absorption maxima, opsin shifts and shape of absorption spectra of artificial and natural pigments are shown in Table 1.

RESULTS AND DISCUSSION

We reported that when the apoprotein from *Halobacterium salinarum* (pR) was used, both 8,18- and 8,16-ethanoretinols (**2** and **3a**) were incorporated to afford the artificial pigments [5]. However, in the case of the apoprotein from ppR, 8,18-ethanoretinol (**2**, 6*s-cis* conformation) was not incorporated in the protein,

[§] Satisfactory ¹H-NMR, IR and MS spectral data were obtained. ¹H-NMR data for compound **3b**: (500 MHz, CDCl₃) δ 1.13 (3H, s, Me), 1.2-1.5 (6H, m, CH₂ × 3), 1.6-1.8 (2H, m, CH₂), 1.74 (3H, s, Me), 1.81 (3H, s, Me), 1.9-2.1 (2H, m, CH₂), 2.3-2.5 (2H, m, CH₂), 5.67 (1H, t, *J*=4, CH), 6.00 (1H, d, *J*=8, CH), 6.06 (1H, d, *J*=15, CH), 6.25 (1H, d, *J*=11, CH), 6.41 (1H, s, CH), 6.85 (1H, dd, *J*=15, 11, CH), 10.03 (1H, d, *J*=8, CHO). For **3c**: (500 MHz, CDCl₃) δ 1.2-1.7 (6H, m, CH₂ × 3), 1.67 (3H, s, Me), 1.7-1.8 (2H, m, CH₂), 1.75 (3H, s, Me), 1.91 (3H, s,

Me), 1.9-2.0 (2H, m, CH₂), 2.3-2.6 (3H, m, CH₂ and CH), 6.01 (1H, d, *J*=8, CH), 6.09 (1H, d, *J*=15, CH), 6.32 (1H, d, *J*=11, CH), 6.77 (1H, s, CH), 6.90 (1H, dd, *J*=15, 11, CH), 10.04 (1H, d, *J*=8, CHO). For **3d**: (500 MHz, CDCl₃) δ 1.4-1.7 (6H, m, CH₂ × 3), 1.7-1.8 (2H, m, CH₂), 1.74 (3H, s, Me), 1.84 (3H, s, Me), 1.9-2.1 (2H, m, CH₂), 2.4-2.6 (3H, m, CH₂ and CH), 5.75 (1H, br t, *J*=4, CH), 6.01 (1H, d, *J*=8, CH), 6.06 (1H, d, *J*=15, CH), 6.29 (1H, d, *J*=11.5, CH), 6.41 (1H, s, CH), 6.87 (1H, dd, *J*=15, 11, CH), 10.04 (1H, d, *J*=8, CHO).

Table 1. Absorption Maxima, Opsin Shifts and Shape of Spectra of Pigments

Chromophore Compds	Aldehyde ^a λ max (nm)	PSB ^b λ max (nm)	ppR ^c λ max / nm	Opsin Shift $\Delta \nu$ / cm^{-1}	Fine Structure
Retinal (1)	381	443	497	2450	+
5-Demethyl-8,16-ethanoretinol (3b)	377	440	470	1450	-
1-Demethyl-8,16-ethanoretinol (3c)	387	462	475	590	-
1,5-Bisdemethyl-8,16-ethanoretinol (3d)	376	436	483	2230	+
8,16-Ethanoretinol (3a)	373	437	472	1700	-
8,18-Ethanoretinol (2)	382	450	_ d	_ d	_ d

^a In ethanol.^b In methanol.^c Generated new pigment.^d No pigment formed.

suggesting that the binding pocket of ppR is more restricted than that of pR, and all the 8,16-ethanoretinols (**3a-d**, 6*s-trans* conformation) combined with apoprotein to produce the new pigments. From these facts, for the first time, the conformation around the 6-7 single bond of retinal chromophore in sRII is chemically substantiated as 6*s-trans* conformation. Among the artificial pigments, a spectral shoulder was only observed in the case of **3d**. From the consideration of both the opsin shift value, the parameter of the interaction between the chromophore and the apoprotein [9], and the shape of absorption spectra, it was found that the conformation of **3d** had a strongest resemblance to that of retinal in the native pigment.

The difference in the spectral shape of analog **3d** from **3a-c** could be explained in terms of the different flexibility of the torsional angle around the C6-C7 single bond. The introduction of methyl substituent at the 5 or 1 position in the structure of **3d** should decrease the flexibility of C6-C7 single bond at the binding site because of the steric bulkiness of the substituent. In such cases, the serious destruction of the coplanarity in the polyolefinic system may arise due to different orientation of methyl group at position 5 from that of the native pigment, thus resulting in the disappearance of the fine structure in the absorption spectrum. This consideration was further supported by the fact that the artificial pigment produced by the retinal analog **15** [10], whose 8 and 16 positions were directly connected and the coplanarity in the polyolefinic system was preserved by the locked structure, showed the fine structure clearly in its absorption spectrum (Fig. 3).

On the contrary, Kamo and co-workers [11] reported that when a retinal analog, replaced by phenyl group from 2,6,6-trimethylcyclohexene ring of retinal, was incorporated in the apoprotein and that the spectral shoulder was not observed in its absorption spectrum. In this case, the planar chromophore phenyl ring will be able to occupy the twisted conformation with respect to the polyolefinic side chain, therefore, the coplanarity of the full conjugated system will be destroyed in the protein pigment.

By combining the above findings, we speculate that not only the coplanarity of the plane formed by the polyolefinic system but also the flexibility of the C6-C7 single bond is

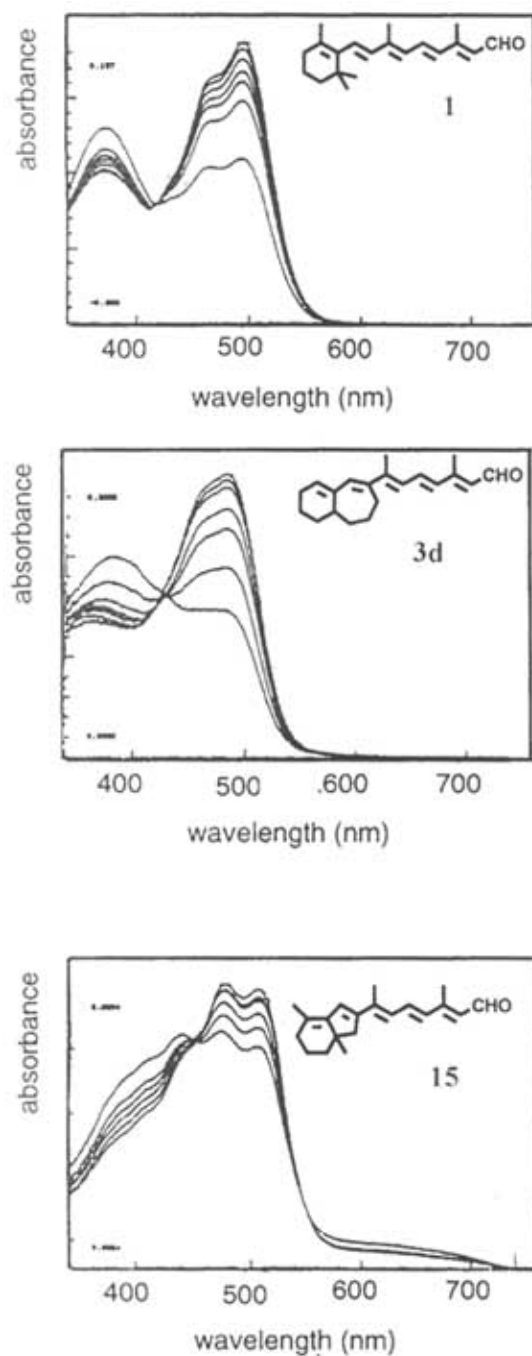


Fig. (3). Absorption spectra of pigments.

important for the appearance of spectral shoulder. Also, it seemed that the range of the torsional angle for exhibiting the fine structure in its absorption spectrum of the pigment is rather limited. A further study is in progress in our laboratory.

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