A NEW RHODOPSIN ANALOG INVOLVING 11Z-8,18-ETHANORETINAL AS A CHROMOPHORE

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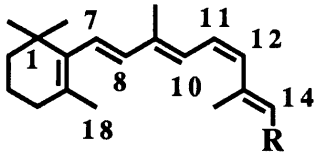
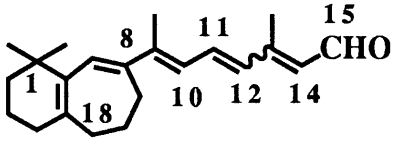
11Z-8,18-Ethanoretinal was synthesized from the β-ionone analog (**5**) *via* the Wittig reaction, and its binding experiment with bovine opsin afforded the new rhodopsin analog, whose opsin shift and CD spectrum were similar to those of the native rhodopsin.

KEYWORDS 8,18-ethanoretinal; rhodopsin analog; CD; opsin shift; retinal analog; rhodopsin

Rhodopsin (**1**) is the visual pigment which contains 11Z-retinal (**2**) as a chromophore bound to the ε–amino group of the apoprotein lysine residue through a protonated Schiff base, and exhibits a characteristic circular dichroism (CD) signal at α and β bands in the visible and near-UV region.[1] During our studies to clarify the photobleaching process of rhodopsin,[2] it was confirmed that the origin of the α-CD band was the torsion around the 12-13 single bond of retinal chromophore.[2c] As an extension of our investigation, we describe here the synthesis of 11Z-8,18-ethanoretinal (**3**), in which C8 and C18 positions in **2** are connected by an ethylene group, and its interaction with bovine opsin, in order not only to investigate the conformation around the cyclohexene ring and polyene side chain in the chromophore, but also to elucidate the origin of the β-CD band.



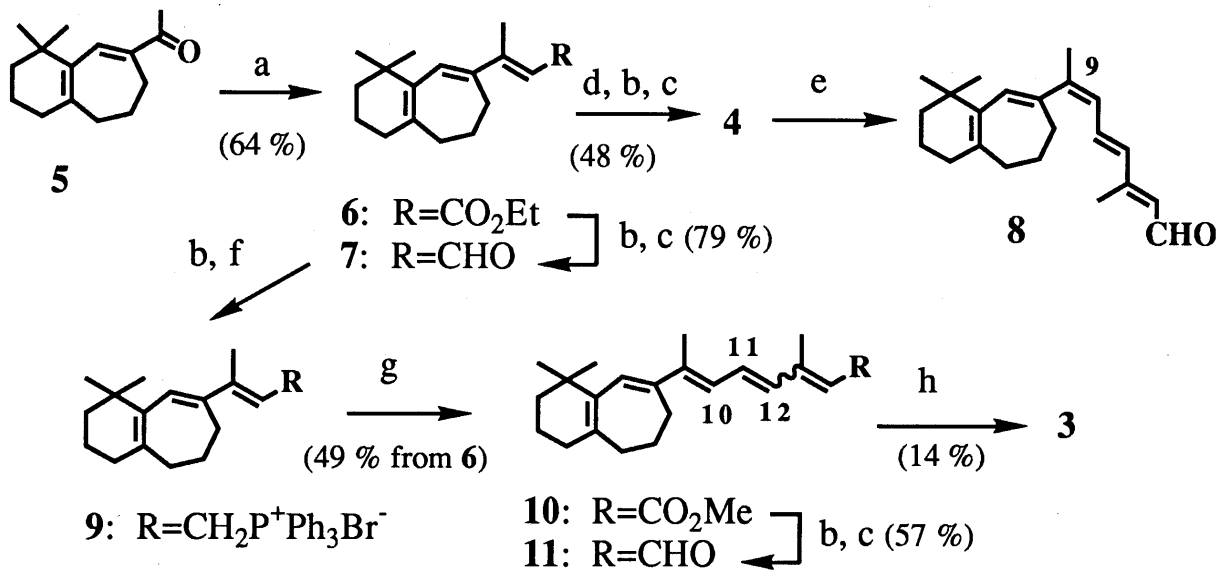
1: R= CH=$\overset{+}{N}$H–opsin
2: R= CHO



3: 11Z
4: All-*E*

It is well known in retinal chemistry that the photochemical isomerization of the all-*E* isomer in polar solvent and subsequent HPLC isolation is the most promising method for preparation of 11Z isomer.[3] Initially we tried the photoisomerization of all-*E*-8,18-ethanoretinal (**4**), which was prepared from 2,2-dimethylcyclohexanone *via* β-ionone analog (**5**).[4] Irradiation of **4** in acetonitrile or methanol under nitrogen using a fluorescent daylight lamp afforded mainly the mixture of all-*E* (**4**) and 9Z (**8**)[5,6] isomers.

Kobayashi et al. reported a high yield synthesis of retinal analog containing 11*Z* isomer in reasonable concentration by the reaction of β-ionylideneacetaldehyde analog with (3-hydroxymethyl-2-methylallyl)-triphenyl phosphonium hydrobromide under the basic conditions and subsequent MnO_2 oxidation.[7] This methodology was applied to the reaction between the aldehyde (**7**) and (3-methoxycarbonyl-2-methylally)-triphenylphosphorane, but no 11*Z* isomer (**3**) was detected. On the contrary, the Wittig reaction of methyl *E*-3-formylcrotonate with phosphonium salt (**9**), which was derived from the ester (**6**) by $LiAlH_4$ reduction followed by treatment with triphenylphosphonium hydrobromide, in the presence of sodium methoxide afforded the pentaene ester (**10**) as a mixture of geometrical isomers [all-*E*:11*Z*=*ca.* 2:1]. Without isolation of this mixture, the transformation of **10** to the corresponding aldehyde mixture (**11**) was achieved according to the usual method by $LiAlH_4$ reduction and MnO_2 oxidation, and the 11*Z* isomer (**3**)[5,8] was isolated in pure form by repeating the preparative HPLC in the dark. As shown in the previous reports,[2a,g] the present method using the Wittig reaction between the analog of β-ionylideneethyltriphenylphosphonium salt and C5 aldehyde provides a fundamentally useful route for the synthesis of 11*Z* isomer of retinal analogs.



a) $NaH,(EtO)_2P(O)CH_2CO_2Et$ / 10% DMF-THF, reflux, b) $LiAlH_4$ / Et_2O, r.t., c) MnO_2 / CH_2Cl_2, r.t., d) *n*-BuLi, $(EtO)_2P(O)CH_2(CH_3)C{=}CHCO_2Me$ / THF, -78°-0°C, e) hν, f) Ph_3P HBr / MeOH; g) NaOMe, $OHC(CH_3)C{=}CHCO_2Me$ / CH_2Cl_2, h) prep. HPLC.

Subsequently, the binding experiment of **3** with bovine opsin was carried out in a CHAPS-PC mixture by the method reported previously[2g] to afford a new artificial rhodopsin having the absorption maximum at 503 nm. The protonated Schiff base (PSB) of **3** with *n*-butylamine was formed by the usual method. The UV-VIS, CD data and opsin shifts of the artificial pigment and native rhodopsin are shown in the Table. All the values of the new rhodopsin analog are very close to those of native rhodopsin. These results strongly suggest that the conformations of the two chromophores are almost the same in the protein.

For the first time the torsional angle around the 6-7 single bond in the rhodopsin chromophore is chemically substantiated using 6s-fixed bicyclic retinal analog (**3**).

Table I. The UV-VIS, CD Data and Opsin Shifts of Rhodopsins.

Chromophores	Aldehydes[a] λ max / nm	PSB[b] λ max / nm	Rhodopsins[c] CD nm λ max / nm	CD nm (mdeg / absorption) α–band	CD nm (mdeg / absorption) β–band	Opsin shifts Δν / cm^{-1}
11Z-8,18-Ethanoretinal (**3**)	386	457	503	491 (+8.23)	335 (+16.97)	2000
11Z-Retinal (**1**)	376.5	440	498	487 (+8.63)	335 (+17.50)	2650

a) In ethanol. b) In methanol . c) In CHAPS-PC mixture.

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

6) ^{1}H-NMR data for compound **8** are as follows: (500 MHz, C_6D_6) δ 1.03 (6H, s, 1-Me), 1.42-1.45 (2H, m, 2-H_2), 1.80 (3H, d, *J*= 1 Hz, 13-Me), 1.82 (3H, br s, 9-Me), 1.92-2.00 (6H, m, 18,18a,18b-H_2), 2.05 (2H, br t, *J* = 6.5 Hz, 4-H_2), 5.84 (1H, d, *J* = 11 Hz, 10-H), 5.99 (1H, d, *J* = 8 Hz, 14-H), 6.05 (1H, d, *J* = 16 Hz, 12-H), 6.08 (1H, br s, 7-H), 7.11 (1H, dd, *J* = 16, 11 Hz, 11-H), 9.99 (1H, d, *J* = 8 Hz, CHO). This structure was determined by its ^{13}C-NMR of methyl signal at position 9, which was exhibited at δ 24.43; see, G. Englert, *Helv. Chim. Acta*, **58**, 2367 (1975).

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8) ^{1}H-NMR data for compound **3** are as follows: (500 MHz, C_6D_6) δ 1.07 (6H, s, 1-Me), 1.42-1.47 (2H, m, 2-H_2), 1.56-1.62 (2H, m, 3-H_2), 1.79 (3H, d, *J*= 1 Hz, 13-Me), 1.86 (3H, br s, 9-Me), 1.88 (2H, t, *J* = 7 Hz, 18-H_2), 2.02-2.12 (2H, m, 4-H_2), 2.05 (2H, quint, *J* = 7 Hz, 18a-H_2), 2.29 (2H, t, *J* = 7 Hz, 18b-H_2), 5.63 (1H, d, *J* = 11.5 Hz, 12-H), 6.16 (1H, br d, *J* = 8 Hz, 14-H), 6.45 (1H, br s, 7-H), 6.47 (1H, t, *J* = 11.5 Hz, 11-H), 6.90 (1H, d, *J* = 11.5 Hz, 10-H), 9.95 (1H, d, *J* = 8 Hz, CH CHO).

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