CHEMISTRY LETTERS, pp. 2005-2008, 1986.

Highly Stereoselective Photoisomerization of all-trans Naphthyl Retinal Analog to the 11-cis Isomer by Protein Mediation

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Protein-mediated photoisomerization of all-trans Np-retinal stereospecifically gave the 11-cis isomer whose structure was determined, whereas irradiation of all-trans Np-retinal in acetonitrile furnished the four isomers, none of which was the 11-cis isomer.

Unlike photochemical isomerization in organic solvents, biochemical photoisomerization is highly stereoselective. It is well-known that rhodopsin found in retina undertakes structural change of a chromophore when irradiated.[1] The change can be depicted as the stereospecific photoisomerization of 11-cis retinal into all-trans retinal. Then the reverse reaction, from all-trans to 11-cis retinal, inevitably can be envisaged. Biochemically the reaction is achieved which has been exemplified by photoreaction of retinochrome in invertebrates.[2] However, it has not been investigated so far whether the similar reaction takes place or not if an analog is used instead of all-trans retinal. The generalization of the reaction would play a crucial role not only in stereospecific photochemistry but also in vision chemistry.

As is well-known, the synthesis of 11-cis retinal is accomplished by photochemical isomerization of all-trans retinal in an organic solvent.[3] The

most effective method for the preparation of 11-cis retinal has been reported that irradiation of an acetonitrile solution of all-trans retinal followed by HPLC isolation gives 11-cis retinal in 45.2% yield on the basis of the isomerized retinals.[4] This reaction seems to be applied to photochemical transformation of the analogs.

Naphthyl analog (Np-retinal) was synthesized as previously reported.[5] Irradiation of an acetonitrile solution of all-trans Np-retinal with 400 nm light gave a mixture of Np-retinal isomers.[6]

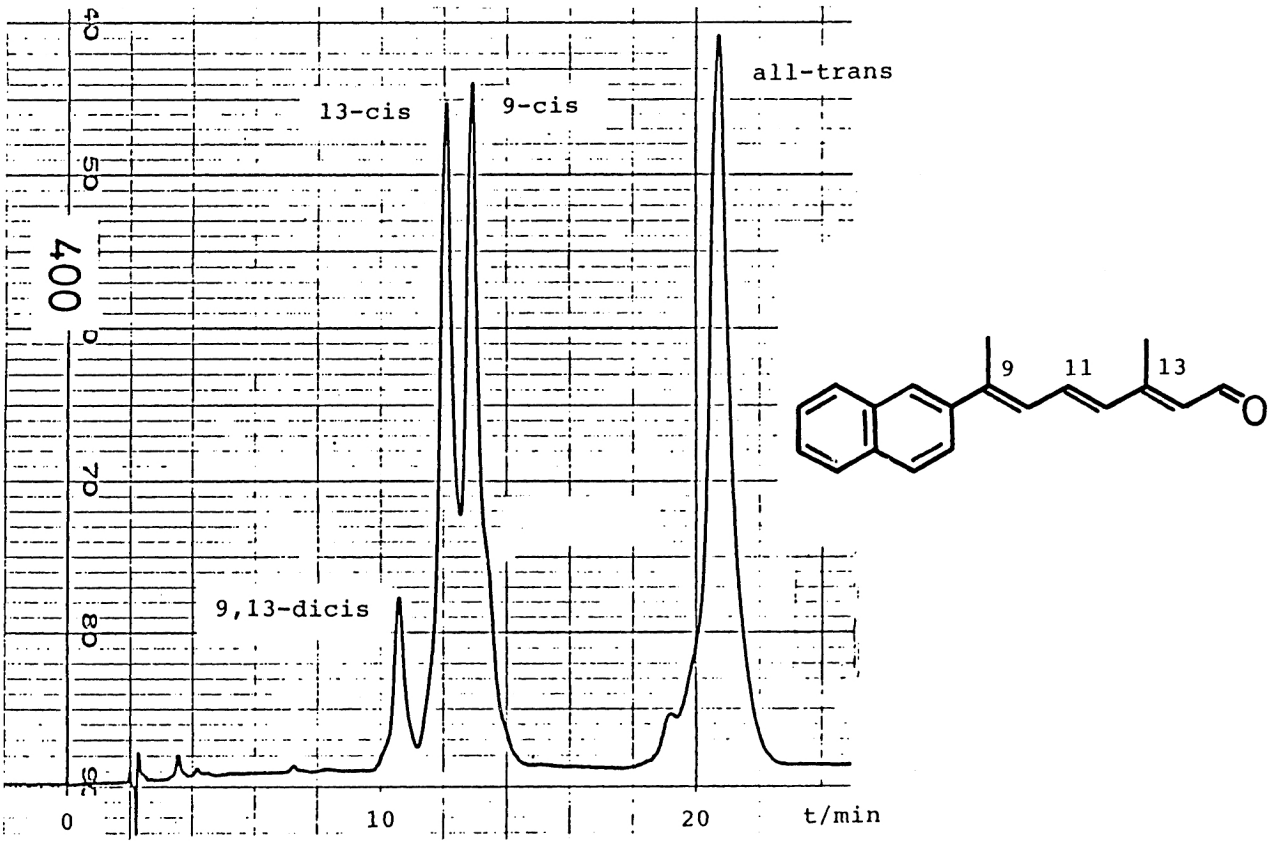

Fig. 1. HPLC of the photoproducts in the photoisomerization of Np-retinal.

A HPLC chart of the mixture is shown in Fig 1. All fractions corresponding to the peaks were collected by the use of HPLC and these structures were determined by proton NMR.[7] The chemical shifts for the olefinic proton and their coupling constants are shown in Table 1. The underlined values indicate anomalous chemical shifts. These shifts can be rationalized by the anisotropy of carbonyl and naphthyl substituents. It is especially different from normal retinal isomers that the naphthyl ring in 9-cis form is perpendicular to the

conjugated chain so that the 11-proton is located at a right angle to the naphthalene plane.

Table 1. Chemical Shifts and Coupling Constants for Aldehydic and Olefinic Protons of Np-retinals(in CD_3COCD_3)

Np-retinal Isomers	Chemical Shifts and Coupling Constants Bracketed[a] 15H	14H	12H	11H	10H
all-trans[b]	10.15 (8)	5.96 (8)	6.67 (15)	7.41 (15,11)	6.94 (11)
13-cis	10.30 (8)	5.83 (8)	7.69 (15)	7.29 (15,11)	6.69 (11)
9-cis	10.06 (8)	5.89 (8)	6.51 (15)	6.96 (15,11)	6.48 (11)
9,13-dicis	10.21 (8)	5.71 (8)	7.48 (15)	6.82 (15,11)	6.48 (11)
11-cis	10.13 (8)	6.00 (8)	6.19 (12)	6.87 (12,12)	--[c]
11-cis retinal[b,7]	10.10 (8)	6.07 (8)	5.92 (12)	6.69 (13,12)	6.54 (13)

a) δ/ppm and J/Hz. b) In $CDCl_3$. c) Overlapped with contaminant peaks.

In the photoisomerization of all-trans Np-retinal, four isomers were isolated as products; 9,13-dicis, 13-cis, 9-cis,and all-trans isomers, whose products ratios were 5, 25, 26,and 44%, respectively. The 11-cis isomer could not be isolated because the amount was too small. This fact is in stark contrast to the case in retinal photochemistry. There is no stereochemical limitation on formation of the 11-cis isomer in considering the molecular structure with a space-filling model.

In order to carry out protein-mediated photoisomerization, we have isolated retinochrome from squid eyes according to Hara's method.[2] The isolated protein was identified as retinochrome by the absorption spectra (λ_{max}/nm 495), decolorization with hydroxylamine and the electrophoresis (24 kdalton). After retinochrome was converted into apo-retinochrome which consists of the same protein but does not include the retinal molecule,[2] the apo-retinochrome was bound to the Np-retinal above-mentioned by mixing equimolarly. The formed pigment showed the absorption maximum at 458 nm, which is totally identical with the previously reported value.[8]

The pigment was irradiated with light at 550 nm avoiding reabsorption of light by the produced retinal. Subsequent hexane extraction of the photoisomers

gave a single isomer, whose HPLC showed that the retention time was 14 min under the same conditions above-mentioned. The structure of the isomer was determined based on the analyses of the 270 MHz NMR spectra and the mass spectra ($M^{+\cdot}$ m/z 262). The olefinic proton signals of the NMR spectra especially should give decisive information on the geometrical isomers. A doublet signal at 6.00 ppm (J=8 Hz) coupled with the doublet aldehydic signal at 10.13 ppm (J=8 Hz) is assigned to the 14H. A triplet signal (J=12 Hz) at 6.87 ppm is characteristic and assigned to 11H, which suggests that the isomer has the 11-cis structure.

In conclusion protein-mediated photoisomerization of all-trans Np-retinal stereospecificallly gave the 11-cis isomer, whereas irradiation of all-trans Np-retinal in acetonitrile produced the four isomers, none of which was the 11-cis isomer. We were able to isolate the 11-cis isomer for the first time by the protein-mediated photoisomerization. This method is so promising for stereospecific photoisomerization that formation of artificial rhodopsin analogs consisting of the 11-cis-typed retinal structure is now in progress.

We thank Mr. Osamu Ohtsuka at the Niigata fisheries experiment station for his furnishing the squids for this study. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

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(Received September 2, 1986)