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Formation of 7-*Cis* Retinal by the Direct Irradiation of All-*Trans* Retinal[1]

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7-*cis* Retinal, one of the geometrical isomers of retinal, was prepared by the direct irradiation of all-*trans* retinal dissolved in ethanol and successive separation by high performance liquid chromatography. The procedures for its purification and identification are described.

High performance liquid chromatography (HPLC) has recently been used for the analysis and purification of geometrical isomers of retinal (*1-8*). By means of this technique, we have also studied the isomeric composition of retinal from bacteriorhodopsin (*7*) and some photoproducts of frog rhodopsin (*8*) and squid rhodopsin (unpublished results). In the course of these studies, many opportunities have arisen to prepare authentic isomers by HPLC separation from irradiated products of all-*trans* retinal. However, we observed a small peak in addition to the retinal isomers which have so far been reported to be present in these products (*3*, *4*, *9*). This was identified as 7-*cis* retinal as described below.

7-*cis* Retinal was first synthesized by the group of Liu (*6*, *10*, *11*) by a combination of sensitized irradiation with highly refined procedures of organic synthesis. They also argued (*10*) that 7-*cis* retinal is not directly produced by the irradiation of all-*trans* retinal.

Interestingly, DeGrip *et al.* (*12*) reported that 7-*cis* retinal forms a photosensitive pigment with cattle opsin. Recently we identified this isomer as the chromophore in a photoproduct of squid rhodopsin (unpublished results). Therefore, the simple procedure for obtaining 7-*cis* retinal described below should be useful for studies of rhodopsin photochemistry.

MATERIALS AND METHODS

Retinal—Both all-*trans* and 9-*cis* retinals were purchased from Sigma. 11-*cis* Retinal was a gift from Mr. Paul Brown of Harvard University. 13-*cis* Retinal was prepared from the irradiated products of all-*trans* retinal in hexane (Wako, Spectral grade) (*7*, *9*). Each isomer was purified by HPLC. Identification of these isomers was carried out by comparison of their spectra with those described in the literature (*13*).

Irradiation—All-*trans* retinal solution in a glass vessel was cooled to 0°C in an ice bath and irradiated with white light from a projector equipped with a 1 kW tungsten lamp. No special care was taken as regards deaeration or the selection of excitation wavelengths.

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Abbreviation: HPLC, high-performance liquid chromatography.

HPLC—HPLC was carried out in a JASCO FLC-350 unit equipped with a silica column (JASCOPACK SS-05-250). The sample in 25 μl of heptane (Nakarai, Spectral grade) was injected by the stopped flow technique. Solvent, petroleum ether (bp 30°–50°C): diethyl ether=92 : 8 (v/v). Flow rate, 2 ml/min. Chromatography was monitored through a flow cell using a JASCO UVIDEC-100 spectrophotometer.

Spectroscopy—For measurements of spectra or for application to the second chromatography, peak fractions were dried under a stream of nitrogen and then dissolved in a small volume of heptane (Nakarai, Spectral grade). Absorption spectra were recorded on a Hitachi 323 recording spectrophotometer.

Opsin Test—Cattle opsin was prepared as described by Hubbard *et al.* (*14*) and finally dissolved in 2% digitonin solution in 67 mM phosphate buffer, pH 7.1. The opsin solution was added to the dried retinal sample. The mixture was agitated and then incubated at 34°C for 16 h. Hydroxylamine solution (neutralized to pH 7.1 with KOH just before use) was then added to a final concentration of 0.1 M. After recording the whole spectrum, the sample was irradiated with white light until the yellow colour was completely bleached. The spectrum of the bleached sample was recorded. All of the above manipulations were done in the dark unless otherwise stated.

RESULTS AND DISCUSSION

Figure 1 shows the HPLC pattern of the products obtained by irradiating all-*trans* retinal dissolved in ethanol (Nakarai, guaranteed grade. No further purification was carried out.). From a comparison of the retention times and the absorption spectra of the eluted samples with those of authentic samples, Peaks 1, 3, 4, and 6 were unambiguously identified as 13-*cis*, 11-*cis*, 9-*cis*, and all-*trans* retinal, respectively. The order of elution in HPLC is consistent with that described by other authors (*1*, *4*) using different HPLC systems. Peaks 1, 3, 4, and 6 attained photoequilibrium within a short period (about 30 min) of irradiation, while Peak 2 increased progressively. The component in Peak 2 has not been well characterized, because this fraction gave a strong UV absorption.

A trace peak between 9-*cis* retinal and all-*trans* retinal, Peak 5, was collected from several runs and subjected to a second chromatography. This, giving a single peak, was again collected and the absorption spectrum was determined (Fig. 2). The maximum was at 359 nm and a small peak characteristic of *cis*-isomers was detected around 250 nm. These features are characteristic of 7-*cis* retinal as described by DeGrip *et al.* (*12*). Furthermore, the retention time of this peak in HPLC coincided with that of authentic 7-*cis* retinal, which was kindly provided by Dr. Liu of Hawaii University.

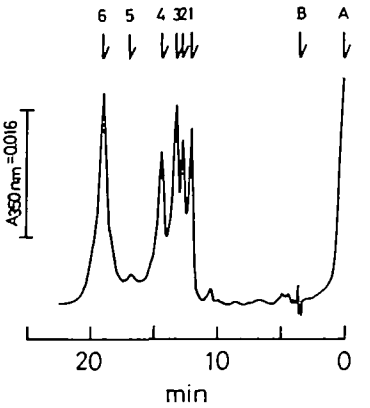

Fig. 1. HPLC pattern of retinal isomers produced by the irradiation of all-*trans* retinal in ethanol. Details are given in " MATERIALS AND METHODS." Peaks, 1, 3, 4, 5, and 6 were assigned as 13-*cis*, 11-*cis*, 9-*cis*, 7-*cis*, and all-*trans* retinal, respectively. Arrows A and B, indicate injection and the solvent front, respectively.

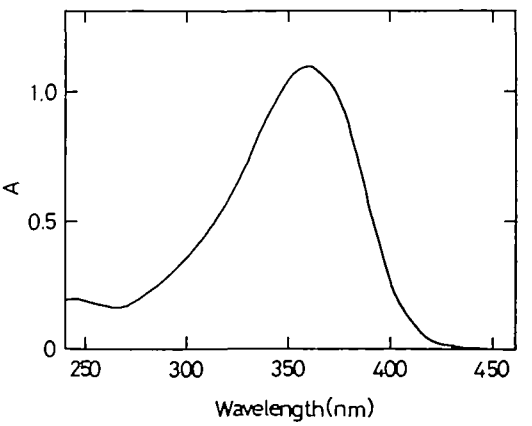

Fig. 2. Absorption spectrum of Peak 5 in Fig. 1. Experimental details are given in the text.

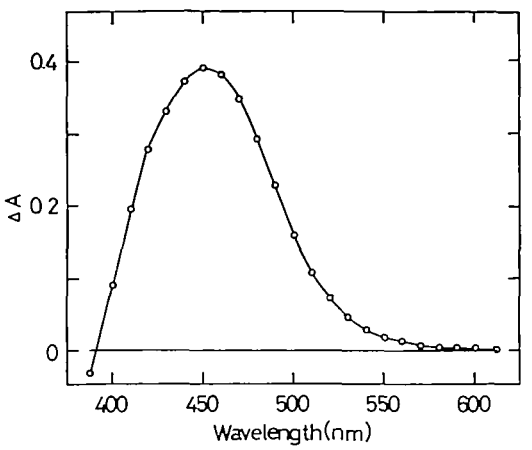

Fig. 3. Absorption spectrum of the complex of Peak 5 with cattle opsin. Details are given in the text. This opsin solution gave $A_{500\,nm}=0.78$ when an excess of 11-*cis* retinal was added.

To confirm that this was the 7-*cis* retinal isomer, the spectrum of the complex produced by the addition of this fraction to cattle opsin was examined. The collected fraction was completely dried and cattle opsin in digitonin solution was added to the dried sample. A slow increase of the absorbance in the visible region due to the formation of 7-*cis* retinal-opsin complex was observed, as described by DeGrip *et al.* (*12*). Figure 3 shows the difference spectrum of the complex (the spectrum before bleaching minus that after bleaching in the presence of hydroxylamine) after incubation for 16 h. The difference spectrum thus obtained gave a maximum at about 450 nm and a band width at half-maximum of about 85 nm, which also coincide with those described by DeGrip *et al.* (*12*). These results unequivocally indicate that the small peak found in HPLC is identical with the material identified as highly hindered 7-*cis* retinal by the group of Liu (*6*, *11*). Thus this isomer, which may be useful as a probe for studies of the interaction between opsin and retinal isomers, can be easily prepared by simply irradiating all-*trans* retinal with successive separation by HPLC.

This isomer was hardly present in the irradiated products of all-*trans* retinal in hexane. As ethanol is much more polar than hexane, the formation of 7-*cis* retinal may be dependent on the polarity of the solvent used for the irradiation process. In this respect, it is interesting to note that the yield of 7-*cis* retinal increased somewhat when irradiation was carried out in methanol (dielectric constant of methanol at 25°C is 32.6, while that of ethanol is 24.3). A similar result was also obtained by Liu using another polar solvent, acetonitrile (personal communication).

It seems likely that previous failures to observe the presence of the 7-*cis* isomer in the irradiated products were due to the difficulty of detecting it. The present finding owes a great deal to the recent development of highly sensitive HPLC systems and the successful chemical synthesis of 7-*cis* retinal by the group of Liu (*6*, *10*, *11*), which afforded invaluable data on the optical properties of this isomer.

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