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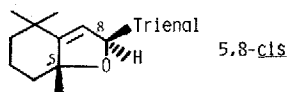
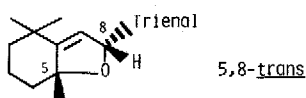
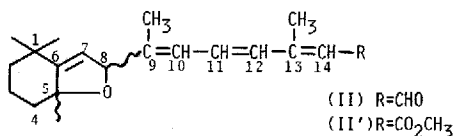
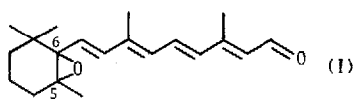
### High-performance liquid chromatographic separation and nuclear magnetic resonance identification of stereoisomers of 5,8-furanoidal retinal\*

MASAYOSHI ITO\*, TAKAKO YAMANE and KIYOSHI TSUKIDA

Kobe Women's College of Pharmacy, 4-19-1 Motoyamakita-machi Higashinada-ku, Kobe 658 (Japan)

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In previous papers<sup>1,2</sup> we have presented the formation of 5,6-epoxyrhodopsin, and the spectral characterisation and high-performance liquid chromatography (HPLC) of geometrical isomers of 5,6-epoxyretinal (I), aiming at a clarification<sup>3</sup> of the protein-chromophore interaction in the rhodopsin molecule. In order to advance our studies, we have prepared a stereochemically proved specimen of 5,8-furanoidal retinal isomer, which is a structural isomer of 5,6-epoxyretinal. The 5,8-oxide (II)<sup>4</sup>,



prepared from all-(*E*)-5,6-epoxyretinal (I) by acid treatment, is a dihydrofuranoid compound with two chiral centres at C-5 and C-8 and a conjugated trienal chain at C-8. Therefore, 5,8-furanoidal retinal should be expected to have several stereoisomers (diastereoisomers and geometrical isomers) though there has been little discussion of them. In addition, no stereoisomer of II has been isolated in a pure form. This paper describes the HPLC separation and proton nuclear magnetic resonance (<sup>1</sup>H NMR) identification of stereoisomers of 5,8-furanoidal retinal (II).

\* Retinoids and related compounds, Part VI. For Part V, see ref. 2.

TABLE I

<sup>1</sup>H NMR DATA\* OF 5,8-*trans*-II AND 5,8-*cis*-II ISOMERS OF ALL-(*E*)-5,8-FURANOIDAL RETINAL

	5,8- <i>trans</i> -II	5,8- <i>cis</i> -II
gemCH <sub>3</sub>	1.11 (s)	1.11 (s)
	1.16 (s)	1.18 (s)
13-CH <sub>3</sub>	2.31 (s)	2.30 (s)
9-CH <sub>3</sub>	1.81 (s)	1.85 (s)
5-CH <sub>3</sub>	1.44 (s)	1.47 (s)
15-H	10.11 (d, <i>J</i> = 8)	10.10 (d, <i>J</i> = 8)
14-H	5.96 (d, <i>J</i> = 8)	5.96 (d, <i>J</i> = 8)
12-H	6.34 (d, <i>J</i> = 15.5)	6.33 (d, <i>J</i> = 15)
11-H	7.01 (dd, <i>J</i> = 11,15.5)	7.00 (dd, <i>J</i> = 11,15)
10-H	6.26 (d, <i>J</i> = 11)	6.26 (d, <i>J</i> = 11)
8-H	5.17 (br.s)	5.07 (br.s)
7-H	5.19 (br.s)	5.25 (d, <i>J</i> = 2)

\* Chemical shift in  $\delta_{\text{ppm}}$ , coupling constant in Hz.

## EXPERIMENTAL

*Materials*

All-(*E*)-5,8-furanoïdal retinal (II) was prepared from all-(*E*)-5,6-epoxyretinal (I) according to the literature<sup>4</sup>. Its two diastereoisomers (5,8-*trans*-II and 5,8-*cis*-II) were separated by low-pressure liquid chromatography (LPLC) followed by HPLC (method 1) and both showed satisfactory evidence of the all-(*E*)-trienal moiety (Tables I and II). Geometrical isomers [11-(*Z*)-5,8-*trans*-II, 13-(*Z*)-5,8-*trans*-II, and 13-(*Z*)-5,8-*cis*-II] were obtained from the irradiated mixture of II (method A or B) by preparative thin-layer chromatography (TLC) followed by HPLC (method 2). Because chromatographic runs were repeated and each peak was collected at the outlet of the detector, all specimens obtained here were well characterised spectroscopically

TABLE II

CHARACTERISTIC <sup>13</sup>C NMR DATA\* OF ALL-(*E*)-5,8-*trans*-II AND 5,8-*cis*-II ISOMERS OF FURANOIDAL RETINAL

	All-( <i>E</i> )- <i>trans</i> -II	All-( <i>E</i> )- <i>cis</i> -II	$\Delta\delta$ <i>trans</i> - <i>cis</i>	$\Delta\delta^{**}$ (refs. 6 and 7)
7-C	118.2	117.1	1.1	1.1
8-C	87.1	87.5	-0.4	-0.6
9-C	144.7	145.3	-0.6	-0.5
10-C	125.4	124.2	1.2	0.9
11-C	132.0	132.2	-0.2	0
12-C	134.9	134.7	0.2	0.4
9-CH <sub>3</sub>	13.1	13.8	-0.7	-0.4

\* Chemical shift in  $\delta_{\text{ppm}}$  obtained from the mixture of 5,8-*trans*- and 5,8-*cis*-II.\*\*  $\Delta\delta$  = (flavoxanthin - chrysanthemaxanthin).

TABLE III

<sup>1</sup>H NMR DATA\* OF 11-(Z)-5,8-*trans*-II, 13-(Z)-5,8-*trans*-II, and 13-(Z)-5,8-*cis*-II

	11-(Z)- 5,8- <i>trans</i> -II	13-(Z)- 5,8- <i>trans</i> -II	13-(Z)- 5,8- <i>cis</i> -II
gemCH <sub>3</sub>	1.11 (s)	1.11 (s)	1.12 (s)
13-CH <sub>3</sub>	1.16 (s)	1.16 (s)	1.19 (s)
9-CH <sub>3</sub>	2.33 (s)	2.12 (s)	2.13 (s)
5-CH <sub>3</sub>	1.76 (s)	1.81 (s)	1.86 (s)
	1.43 (s)	1.44 (s)	1.47 (s)
15-H	10.06 (d, <i>J</i> = 8)	10.19 (d, <i>J</i> = 8)	10.19 (d, <i>J</i> = 8)
14-H	6.01 (d, <i>J</i> = 8)	5.87 (d, <i>J</i> = 8)	5.84 (d, <i>J</i> = 8)
12-H	5.92 (d, <i>J</i> = 11)	7.28 (d, <i>J</i> = 15)	7.23 (d, <i>J</i> = 15)
11-H	6.52 (t, <i>J</i> = 11)	6.92 (dd, <i>J</i> = 11,15)	6.89 (dd, <i>J</i> = 11.5,15)
10-H	6.64 (d, <i>J</i> = 11)	6.32 (d, <i>J</i> = 11)	6.28 (d, <i>J</i> = 11.5)
8-H	5.15 (br.s)	5.18 (br.s.)	5.09 (br.s.)
7-H	5.17 (br.s)	5.19 (br.s)	5.26 (d, <i>J</i> = 2)

\* Chemical shift in  $\delta_{\text{ppm}}$ , coupling constant in Hz.

(Tables I-IV) and their molecular formulae were determined by high-resolution measurement of molecular weight (Table V).

#### Photoisomerisation procedure

*Method A.* A solution of all-(*E*)-5,8-*trans*-II (10 mg) in methanol (10 ml) was stirred in a flask and exposed to the light from a fluorescent lamp (30 W) at a distance of 10 cm for 3 h.

*Method B.* A solution of all-(*E*)-5,8-furanoidal retinal (a mixture of 5,8-*trans*-II and *cis*-II) (50 mg) in methanol (50 ml) was irradiated using a high-pressure mercury lamp (300 W, EIKOSHA Co.) through a Pyrex filter under bubbling nitrogen for 5 h.

TABLE IV

UV SPECTRAL DATA [ $\lambda_{\text{max}}$ (nm) AND  $\epsilon$ ( $\text{lmol}^{-1} \text{cm}^{-1}$ )] OF 5,8-FURANOIDAL RETINALS

	In ethanol	In <i>n</i> -hexane
All-( <i>E</i> )-5,8- <i>trans</i> -II	331 ( $\epsilon = 36,700$ )	303(sh) ( $\epsilon = 22,100$ ) 317 ( $\epsilon = 33,200$ ) 332 ( $\epsilon = 30,800$ )
11-(Z)-5,8- <i>trans</i> -II	229 328	
13-(Z)-5,8- <i>trans</i> -II	231 ( $\epsilon = 11,300$ ) 330 ( $\epsilon = 30,200$ )	231 ( $\epsilon = 9700$ ) 303(sh) ( $\epsilon = 23,300$ ) 317 ( $\epsilon = 32,000$ ) 331 ( $\epsilon = 28,400$ )
13-(Z)-5,8- <i>cis</i> -II	232 328	

TABLE V

HIGH-RESOLUTION MEASUREMENT OF MOLECULAR WEIGHT OF FURANOIDAL RETINALS; MOLECULAR FORMULA  $C_{20}H_{28}O_2$ 

	<i>Calcd.</i>	<i>Found</i>
All-( <i>E</i> )-5,8- <i>trans</i> -II	300.209	300.210
All-( <i>E</i> )-5,8- <i>cis</i> -II	300.209	300.209
11-( <i>Z</i> )-5,8- <i>trans</i> -II	300.209	300.211
13-( <i>Z</i> )-5,8- <i>trans</i> -II	300.209	300.208
13-( <i>Z</i> )-5,8- <i>cis</i> -II	300.209	300.209

### Spectra

Ultraviolet (UV) spectra were recorded on a Shimadzu 200S instrument. All NMR spectra were measured on a Varian XL-200 superconducting FT-NMR spectrometer using solutions in  $C^2HCl_3$ . Mass spectra (MS) were determined on a JEOL JMS-O1SG mass spectrometer; high-resolution measurements were made relative to perfluorokerosene as a reference.

### Chromatography

LPLC was carried out using a Lobar column (Merck LiChroprep Si 60, size C,  $44 \times 3.7$  cm I.D.) connected to a Duramat pump (mobile phase; acetone-*n*-hexane, 10:90).

HPLC was carried out on a Shimadzu LC-3A or LC-2 chromatograph, equipped with UV-detector. Method 1: stationary phase, LiChrosorb Alox T ( $30 \times 0.4$  cm I.D.); mobile phase, diethyl ether-*n*-hexane, (20:80); pressure, 60 kg/cm<sup>2</sup>; wavelength of analysis, 254 nm. Method 2: stationary phase,  $\mu$ Porasil ( $30 \times 0.4$  cm I.D.); mobile phase, diethyl ether-*n*-hexane, (20:80); pressure or flow-rate, 30 kg/cm<sup>2</sup> or 1.4 ml/min; wavelength of analysis, 330 nm.

Preparative TLC was carried out on a precoated silica gel plate (Merck silica gel 60F<sub>254</sub>, 0.25 or 0.5 mm thickness) developed with diethyl ether-*n*-hexane, (20:80).

## RESULTS AND DISCUSSION

### HPLC separation

The chromatographic separation of the two diastereoisomers (5,8-*trans*-II and 5,8-*cis*-II) of all-(*E*)-5,8-furanoidal retinal was accomplished using LiChrosorb Alox T (alumina system) as the stationary phase and 20% diethyl ether in *n*-hexane as the mobile phase. Fig. 1 shows that complete separation of all-(*E*)-5,8-*trans*-II and its 5,8-*cis*-isomer was achieved, the latter having the longer retention time. The isomer-ratio of the products prepared by acid-catalysed rearrangement of all-(*E*)-5,6-epoxyretinal (I) has been determined to be *ca.* 3:1 (predominantly all-(*E*)-5,8-*trans*-II) from the peak height by application of the analytical condition mentioned above. No satisfactory separation of the two diastereoisomers (5,8-*trans*-II and 5,8-*cis*-II) of all-(*E*)-5,8-furanoidal retinal was achieved using  $\mu$ Porasil (silica system), although several different solvent systems were tested.

The chromatographic separation of *E*-*Z* isomers in a mixture obtained by photoisomerisation of all-(*E*)-5,8-*trans*-II has been accomplished using  $\mu$ Porasil

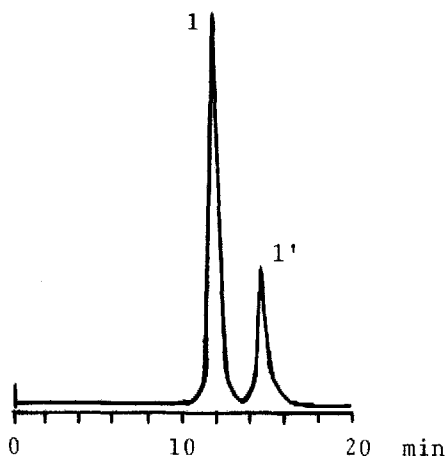


Fig. 1. HPLC chromatogram of a mixture of two furanoidal diastereomers. Peaks: 1 = all-(*E*)-5,8-*trans*-II; 1' = all-(*E*)-5,8-*cis*-II. LiChrosorb Alox T; diethyl ether-*n*-hexane (20:80); 60 kg/cm<sup>2</sup>.

(silica system) as the stationary phase and diethyl ether-*n*-hexane (20:80) as the mobile phase. Two new geometrical isomers, 11-(*Z*)-5,8-*trans*-II and 13-(*Z*)-5,8-*trans*-II, were isolated from the mixture. A typical HPLC chromatogram is shown in Fig. 2, and that of *E*-*Z* isomers obtained by photoisomerisation of a mixture of 5,8-*trans*-II and *cis*-II is shown in Fig. 3. An additional new isomer, 13-(*Z*)-5,8-*cis*-II, has been isolated from the mixture. Attempts to separate the geometrical isomers of 5,8-furanoidal retinal (II) using LiChrosorb Alox T (alumina system) were unsuccessful. From these results, it is evident that an alumina column is appropriate for the separation of stereoisomers at C-5 and C-8, and a silica column for the separation of geometrical isomers in the side-chain.

#### Identification

<sup>1</sup>H NMR spectra of the two compounds (5,8-*trans*-II and 5,8-*cis*-II) produced by acid rearrangement of all-(*E*)-5,6-epoxyretinal (I) were almost identical except for both the chemical shifts and the signal splitting pattern at 7-H and 8-H. Therefore, the configuration of the trienal chain was assumed to be the same in both isomers. It was determined to be all-(*E*)-trienal from both the chemical shift of 13-methyl signal and coupling constant of 11-H signal in the <sup>1</sup>H NMR data (Table I) and the characteristic <sup>13</sup>C NMR data around the C-9-C-10 double bond, in comparison with the <sup>1</sup>H NMR data in all-(*E*)-retinal<sup>5</sup> and the <sup>13</sup>C NMR data in all-(*E*)-5,8-furanoid carotenoids<sup>6,7</sup> and (II)<sup>8</sup>. In addition, the relative conformations of C-5 and C-8 in the two isomers were decided by the application of the method proposed by Cadosch *et al.*<sup>6,7</sup>. From extensive <sup>1</sup>H NMR investigation (chemical shifts at 7-H and 8-H, nuclear Overhauser effect, and aromatic solvent induced shifts of the 5,8-furanoid carotenoids, they concluded that the relationship of the C-8 polyene chain and C-5 methyl on the dihydrofuran ring is *cis* for the pair of stereoisomers having a  $\Delta\delta = \delta(7\text{-H}) - \delta(8\text{-H}) = 0.22$  ppm while the *trans* isomers are  $\Delta\delta = 0.07$  ppm. Characteristic <sup>1</sup>H NMR data, including chemical shifts of 7-H and 8-H in the two stereoisomers (5,8-*trans*-II and 5,8-*cis*-II), are summarised in Table I. The isomer having a  $\Delta\delta = \delta(7\text{-H})$

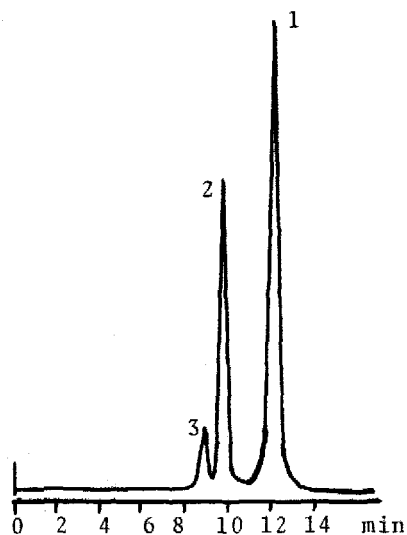


Fig. 2. HPLC chromatogram of an irradiated mixture of all-(*E*)-5,8-*trans*-II. Peaks: 1 = all-(*E*)-5,8-*trans*-II; 2 = 13-(*Z*)-5,8-*trans*-II; 3 = 11-(*Z*)-5,8-*trans*-II. Conditions:  $\mu$ Porasil; diethyl ether-*n*-hexane (20:80); 1.4 ml/min.

—  $\delta(8\text{-H}) = 0.02$  ppm was assigned to be 5,8-*trans*-II and the other isomer showing a  $\Delta\delta = 0.18$  ppm was confirmed to be 5,8-*cis*-II.

The structures of the three new geometrical isomers have been established on the basis of their spectral data (Tables III–V) as 11-(*Z*)-5,8-*trans*-II, 13-(*Z*)-5,8-*trans*-II, and 13-(*Z*)-5,8-*cis*-II, respectively; *i.e.*, a *Z*-geometry of the 11,12-double bond in

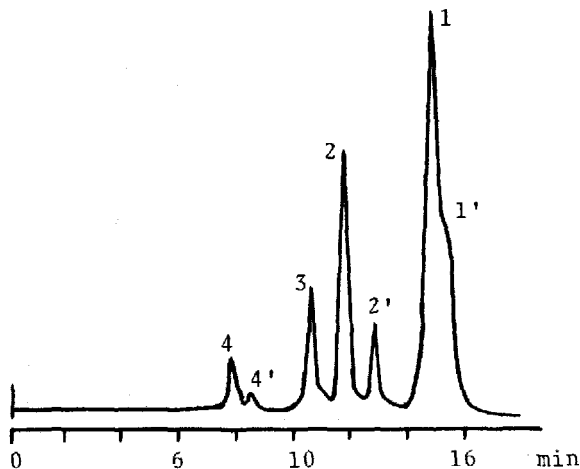


Fig. 3. HPLC chromatogram of an irradiated mixture of the products prepared from 5,6-epoxy-retinal by acid treatment. Conditions:  $\mu$ Porasil; diethyl ether-*n*-hexane (20:80); 30 kg/cm<sup>2</sup>. Peaks: 1 = all-(*E*)-5,8-*trans*-II; 2 = 13-(*Z*)-5,8-*trans*-II; 3 = 11-(*Z*)-5,8-*trans*-II; 1' = all-(*E*)-5,8-*cis*-II; 2' = 13-(*Z*)-5,8-*cis*-II. Peaks 4 and 4' were believed to be the 11,13-di-(*Z*)-5,8-*trans*- and the 11,13-di-(*Z*)-5,8-*cis*-isomers, respectively.

the isomer (11-(Z)-5,8-trans-II) was decided from its coupling constant ( $J_{11,12} = 11$  Hz) and the downfield shift of the signal of 10-H, and a Z-configuration in the isomers [13-(Z)-5,8-trans-II and 13-(Z)-5,8-cis-II] was determined from the downfield shift of the signal of 12-H and the upfield shift of the signal of 13-CH<sub>3</sub> compared with the <sup>1</sup>H NMR data of all-(E)-5,8-trans-II and 5,8-cis-II.

## REFERENCES

- 1 M. Ito, A. Kodama, M. Murata, M. Kobayashi, K. Tsukida, Y. Shichida and T. Yoshizawa, *J. Nutr. Sci. Vitaminol.*, 25 (1979) 343.
- 2 M. Ito, A. Kodama, T. Yamane and K. Tsukida, *J. Nutr. Sci. Vitaminol.*, submitted for publication.
- 3 M. Ito, A. Kodama, K. Tsukida, Y. Fukada, Y. Shichida and T. Yoshizawa, *Chem. Pharm. Bull.*, 30 (1982) 1913, and references cited therein.
- 4 F. B. Jungalwala and H. R. Cama, *Biochem. J.*, 95 (1965) 17.
- 5 V. Ramamurthy and R. S. H. Liu, *Tetrahedron*, 31 (1975) 201.
- 6 H. Cadosch, U. Vögeli, P. Rüedi and C. H. Eugster, *Helv. Chim. Acta*, 61 (1978) 783.
- 7 H. Cadosch, U. Vögeli, P. Rüedi and C. H. Eugster, *Helv. Chim. Acta*, 61 (1978) 1511.
- 8 G. Englert, *Helv. Chim. Acta*, 58 (1975) 2367.