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# Effects of the terminal-methyl position and of the length of the conjugated chain on the *cis* isomers produced by photo-isomerization: analysis by HPLC and configurational determination by <sup>1</sup>H-NMR spectroscopy of isomeric analogues of retinal and $\beta$ -apo-12'-carotenal

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#### Abstract

An analogue of retinal ( $\beta$ -apo-12'-carotenal), its terminal-methyl group being shifted from the 13 to 14 (from 13' to 14') position, was subjected to direct (iodine-sensitized) photo-isomerization. The configurations of five (seven) isomers of the retinal ( $\beta$ -apo-12'-carotenal) analogue were determined by <sup>1</sup>H-NMR spectroscopy to be all-*trans*, 7-, 9- and 11-mono-*cis* and 9,11-di-*cis* (all-*trans*, 9-, 13- and 13'-mono-*cis* and 9,13-, 9,13'- and 13,13'-di-*cis*). Comparative direct photo-isomerization in acetonitrile of the above parent and analogue aldehydes showed that the kinds and the amounts of *cis* isomer produced in the stationary-state mixtures are drastically affected by the terminal-methyl position and by the length of the conjugated chain. The results are discussed in terms of enhanced polarization upon excitation in the terminal C=O and the neighbouring C=C bonds in the conjugated chain.  $\bigcirc$  1997 Published by Elsevier Science B.V.

Keywords: Photo-isomerization; H-NMR spectroscopy; Terminal-methyl position

#### 1. Introduction

Certain *cis* configurations of retinoids and carotenoids play important roles in their physiological functions: 11-*cis*-retinal is bound to

rhodopsin as protonated Schiff base and the 11cis to all-trans photo-isomerization triggers the primary process in vision [1,2]. 15-cis-spheroidene and 15-cis- $\beta$ -carotene are bound to the photo-reaction centers of a purple bacterium [3] and of spinach photosystems II [4] and I [5]. The 15-cis carotenoids are supposed to be involved in the photo-protective function and to dissipate excess

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Fig. 1. The structures of all-*trans* aldehydes having different lengths of the conjugated chain and different positions of the methyl groups. (a) Retinal ( $C_{20}$  aldehyde), (b) 2,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-2,4,6,8- nonatetraenal ( $C_{20'}$  aldehyde), (c) retinylideneacetaldehyde ( $C_{22}$  aldehyde), (d)  $\beta$ -apo-12'-carotenal ( $C_{25}$  aldehyde) and (e) 3,7,11-trimethyl-13-(2,6,6-trimethyl-1-cyclohexenyl)-2,4,6,8,10,12-tridecahexenal ( $C_{25'}$  aldehyde), and (f)  $\beta$ -apo-8'-carotenal ( $C_{30}$  aldehyde). Fragment structures, i.e. (g) 'parallel-methyl fragment' and 'antiparallel methyl fragment' are also defined with double-bond typifications. Arrows indicate the *cis* configurations so far identified; the dotted arrow in  $C_{25}$  aldehyde indicates that the 13'-*cis* isomer can be produced not by direct photo-isomerization but by I<sub>2</sub>-sensitized photo-isomerization; and the small arrow in  $C_{30}$  aldehyde indicates that the amount the 7-*cis* isomer is very small.

triplet energy transferred from chlorophyll [6,7]. The selection of the 11-*cis* configuration by rhodopsin and of the 15-*cis* configuration by the photo-reaction centers is most probably due to the extremely efficient, one-way isomerization into the all-*trans* configuration [6]. An interesting question to be addressed from this viewpoint is why the 15-*cis* configuration, instead of the 11-*cis* configuration, has been used in the physiological functions of carotenoid.

The 11-*cis* isomer of carotenoid has never been found to be produced from the all-*trans* isomer by either photo- or thermal-isomerization [8]. Zechmeister [9] attributed the fact to the severe steric hindrance in the particular unmethylated-*cis* configuration. However, the 7-*cis* [10] and 11-*cis* [11] isomers of retinal can be produced by photoisomerization of the all-*trans* isomer in acetonitrile. Furthermore, the 7-*cis* isomers can be thermally produced from the all-*trans* isomers in  $\beta$ -carotene [12] and photolytically in  $\beta$ -apo-8'carotenal [13]. Thus, the steric hindrance does not completely explain the absence of the 11-cis configuration in the natural carotenoids.

In relation to a more specific question why the 11-cis isomer can be produced photochemically not in carotenoids but in retinoids, we have been determining the *cis-trans* isomers which can be produced in various aldehydes having a series of different lengths of the conjugated chain. Fig. 1(a), (c), (d) and (f) summarizes the *cis* configurations so far identified (those *cis* configurations are indicated by arrows): the 11-cis isomer has been found in retinal (hereafter called C<sub>20</sub> aldehyde) and in retinylidene aldehyde (C<sub>22</sub> aldehyde) [14], but not in  $\beta$ -apo-8'-carotenal [13]. In a previous investigation, we searched for the 11-cis isomer in  $\beta$ -apo-12'-carotenal (C<sub>25</sub> aldehyde), but we could not find it [15]. A discrete change has been found between C<sub>22</sub> aldehyde having six C=C and one C=O double bonds and C<sub>25</sub> aldehyde having seven C=C and one C=O double bonds. Since all the aldehydes have a similar structure, i.e. the  $\beta$ ionone ring at one end and the carbonyl group at

the other end, a most likely answer to the above question has been the length of the conjugated chain.

Comparison of the aldehyde structures in Fig. 1 shows that a definitive difference between the retinoids (C<sub>20</sub> and C<sub>22</sub> aldehydes) and the apo-carotenals ( $C_{25}$  and  $C_{30}$  aldehyde) lies in the positions of the methyl groups attached to the conjugated chain. The retinoids contain only the  $C(CH_3)=CH CH=CH-C(CH_3)$  structures (hereafter, we call this 'the parallel-methyl fragment'; see Fig. 1)(g)), but the carotenals additionally contain the  $C(CH_3)=CH-CH=CH-CH=C(CH_3)$  structure (we call this 'the antiparallel-methyl fragment'; Fig. 1(h)). Therefore, the terminalmethyl position might affect the presence or absence of the 11-cis configuration. Actually, we have compared the ground-state and the excited-state properties between C<sub>25</sub> and C<sub>25'</sub> aldehydes by means of NMR and resonance-Raman spectroscopies, and found that the position of the terminal-methyl group affects not only the local electronic distribution in the vicinity of the particular methyl group in the ground state, but also the bond order of the entire conjugated chain in both the singlet- and the triplet-excited states [16].

In the present investigation, we first determined the cis isomers of  $C_{20'}$  and  $C_{25'}$  aldehydes produced by direct and I2-sensitized photo-isomerization of the all-trans isomers, respectively. (We used I2-sensitized photo-isomerization for  $C_{25'}$  aldehyde, because the 13'cis isomer of  $C_{25}$  aldehyde could be obtained only by this method.) Second, in order to find how the terminal-methyl position affects the kinds and the amounts of *cis* isomers produced from the all-trans isomers, we compared the isomeric compositions in the photostationary-state mixtures obtained by direct photo-isomerization of the all-trans C20, C20,  $C_{25}$  and  $C_{25^{\prime}}$  aldehydes in acetonitrile. Finally, we tried to explain the results in terms of 'enhanced polarization of double bonds upon excitation' which takes place in the conjugated chain.

#### 2. Experimental

#### 2.1. Synthesis of $C_{20'}$ aldehyde

All-*trans*  $C_{20'}$  aldehyde, i.e. (2E, 4E, 6E, 8E)-2,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)

-2,4,6,8-nonat etraenal, was synthesized by two steps, i.e. (1) preparation of  $C_{20'}$  ethyl ester from all-*trans*- $\beta$ -ionylidene acetaldehyde and (2) its subsequent reduction and oxidation to the aldehyde; we followed the procedures in the previous preparation of  $C_{25'}$  aldehyde [16] with some modifications: (1) *n*-butyl lithium (13.6 mmol) in *n*hexane solution (8.05 ml; concentration, 1.69 mol/l) was added to a solution of ethyl 4-(diethoxyphosphoryl)-2-methylbut-2-enoate (13.6)mmol) in dry diethyl ether (40 ml). After stirring for 20 min, a solution of all-*trans*- $\beta$ -ionylidene acetaldehyde (10.46 mmol) in dry diethyl ether (20 ml) was added dropwise and the mixture was stirred for an additional 1 h. The rest of the procedure was the same as described previously [16] except for the eluent in silica-gel chromatography (here, a 5:95 mixture of diethyl ether and *n*-hexane was used). An isomeric mixture of  $C_{20'}$ ethyl ester (10.12 mmol) was obtained as orange oil (yield, 97%). (2) A solution of  $C_{20'}$  ethyl ester (10.12 mmol) in dry diethyl ether (20 ml) was added dropwise to a suspension of lithium aluminium hydride (10.12 mmol) in dry diethyl ether (50 ml). The rest of the procedure was the same as described previously [16] except for the amount of manganese dioxide (30 g) and the eluent in silicagel chromatography (here, a 15:85 mixture of diethyl ether and *n*-hexane). All-*trans*  $C_{20'}$  aldehyde (8.38 mmol) was obtained as orange solid particles (yield, 83%), whereas its isomeric mixture (1.06 mmol) was obtained as orange oil (10.5%).

## 2.2. Isolation of the cis-trans isomers of $C_{20'}$ and $C_{25'}$ aldehydes and measurements of their electronic-absorption and NMR spectra

A set of cis-trans isomers of  $C_{20'}$  aldehyde were collected by means of high-pressure liquid chromatography (HPLC) from an isomeric mixture which was obtained by direct photo-isomerization (light intensity, 4000 lux) of the all-*trans* isomer in acetonitrile  $(5.0 \times 10^{-5} \text{ M})$  at 0°C for 6 h under N<sub>2</sub> atmosphere. HPLC conditions were as follows: column, 4 mm i.d. × 300 mm for analysis and 8 mm i.d. × 300 mm for sample collection; adsorbate, Daisogel Si sp-60, 5 µm; eluent, 7% diethyl ether in *n*-hexane; flow rate, 1.0 ml min<sup>-1</sup>; and detection, 360 nm (for analysis) and 380 nm (for collection). The purity of each isomer for NMR measurements was estimated (by assuming the same  $\varepsilon$  value at the detection wavelength) as follows: all-*trans*, 99%; 7-*cis*, 100%; 9-*cis*, 98%; 11-*cis*, 100%; and 9,11-*cis*, 96%.

A set of *cis-trans* isomers of  $C_{25'}$  aldehyde were collected by HPLC from an isomeric mixture which was obtained by iodine-sensitized photo-isomerization (light intensity, 2300 lux) of the all-*trans* isomer ( $5.5 \times 10^{-5}$  M) at room temperature for 30 min under N<sub>2</sub> atmosphere. Exactly the same HPLC conditions were used as in the case of  $C_{25}$  aldehyde [15] except for the separation of the first three peaks (here, the eluent, 5% diethyl ether in *n*-hexane was used). The purity of each isomer for NMR measurements was estimated as follows: all*trans*, 100%; 9-*cis*, 97%; 13-*cis*, 94%; 13'-*cis*, 98%; 9,13-*cis*, 98%; 9,13'-*cis*, 88%; and 13,13'*cis*, 93%.

For comparative direct photo-isomerization of  $C_{20}$ ,  $C_{20'}$ ,  $C_{25}$  and  $C_{25'}$  aldehydes in acetonitrile, the following conditions were used: temperature, 0°C; time of irradiation, 6 h; concentration,  $5.0 \times 10^{-5}$  M for  $C_{20}$  and  $C_{20'}$ aldehydes and  $6.0 \times 10^{-5}$  M for  $C_{25}$  and  $C_{25'}$ aldehydes.

Exactly the same apparatus and conditions as used in the previous investigations [15,16] were used for the measurements of electronic absorption and NMR spectra. The electronic absorption spectra of  $C_{20}$  and  $C_{20'}$  aldehydes were recorded in *n*-hexane. The <sup>1</sup>H-NMR spectra of the set of *cis*-*trans* isomers of  $C_{20'}$  and  $C_{25'}$  aldehydes were recorded in chloroform-d<sub>1</sub> (CEA, 99.6%) and benzene-d<sub>6</sub> (CEA, 99.6%), respectively. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of all-*trans*  $C_{20}$  and  $C_{20'}$  aldehydes were also recorded in benzene-d<sub>6</sub> for comparison.

#### 3. Results and discussion

## 3.1. Isolation by HPLC and configurational determination by <sup>1</sup>H-NMR spectroscopy of the cis-trans isomers of $C_{20'}$ aldehyde

Fig. 2(a) shows an HPLC elution profile for the cis-trans isomers of C<sub>20'</sub> aldehyde. Peaks 1, 2, 3, 4 and 6 will be assigned to the 11-cis, 9-cis, 9,11-cis, 7-cis and all-trans isomers (vide infra); Peak 5 is left unknown due to its low yield, and Peak 7 turned out to be a degradation product.  $C_{20}$ aldehyde exhibits the order of elution of the mono-cis and all-trans isomers as follows: 11-cis, 9-cis, 7-cis and all-trans. It is interesting that the order of elution (among the isomers identified) follows the positional order of the *cis* bend from the carbonyl end to the  $\beta$ -ionone end. The order of elution is 13-cis, 11-cis, 9-cis, 7-cis and then, all-trans in  $C_{20}$  [17] and  $C_{22}$  [14] aldehydes. It is 9-cis, 7-cis and all-trans in C<sub>15</sub> and C<sub>17</sub> aldehydes [14], whereas it is 13'-cis, 15-cis, 13-cis, 9-cis and all-*trans* for  $C_{30}$  aldehyde [13]. It is a general trend that the retention time becomes longer when the all-trans fragment having the carbonyl end becomes longer. Most probably, the order is due to the van der Waals interaction between this alltrans fragment and the microscopically-flat surface of the adsorbate, silica-gel.



Fig. 2. The elution profiles of isomeric mixtures of (a)  $C_{20'}$  aldehyde obtained by direct photo-isomerization in acetonitrile, and (b)  $C_{25'}$  aldehyde obtained by I<sub>2</sub>-sensitized photo-isomerization in *n*-hexane, both starting from the all-*trans* isomer.

Table 1

H-H COSY and NOE correlations detected in the 2D spectra of all the all-trans isomer

C <sub>20'</sub> aldehyde	1 Me	2 H	3 H	4 H	5 Me	7 H	8 H	9 Me	10 H	11 H	12 H	13 H	14 Me	15 H
COSY		÷		•		÷	•		٠					
Long - range COSY	÷	•	-•	٠	••	•	٠	-++	<b>→</b>			٠	- • •	•
NOESY	+				•	••	••		••	••	••	••	•	•

Table 1 lists the COSY correlations for all-trans  $C_{20'}$  aldehyde which were used for the assignment of its <sup>1</sup>H signals (hereafter, <sup>1</sup>H will be abbreviated as H); the case of the all-trans isomer is shown, for example. The COSY peaks correlate the neighbouring olefinic Hs, whereas the long-range COSY peaks correlate the methyl H (hereafter, abbreviated as Me) and the neighbouring olefinic Hs. The carbonyl 15H in the low-field region was used to start the set of assignments. Table 2 lists the chemical shifts of the olefinic Hs for this all-trans configuration, which will be used as the reference in the definition of the isomerization shifts (vide infra). The NOE correlations found in the NOESY spectrum (see both Table 1 and Fig. 3(a)) were used to establish the all-trans configuration of this isomer. The correlations between 1Me and 7H as well as between 5Me and 8H guarantee the s-cis configuration around the 6C-7C axis and the rest of the correlations prove the trans-zigzag configuration of the conjugated chain.

Table 2 also lists the chemical shifts of the olefinic Hs for the set of *cis* isomers. (The H signals were assigned by the use of COSY correlations as in the case of the all-*trans* isomer.) 'The isomerization shifts', which are defined by the changes in the H chemical shifts on going from the all-*trans* isomer to a particular *cis* isomer, are listed in parentheses; larger shifts are underlined. They have been used for configurational determination of the *cis* isomers on the following basis: when a *cis* configuration is introduced, the high-field-shifts (hfs) take place in the convex side due to decrease in the steric interaction between Hs and the low-field-shifts (lfs) take place on the

concave side due to increase in this interaction. Each peak can be assigned as follows: Peak 4 can be assigned to the 7-*cis* isomer because of the hfs of 7H; Peak 2 can be assigned to the 9-*cis* isomer on the basis of the hfs of 10H and the lfs of 8H and 11H; Peak 1 can be assigned to the 11-*cis* isomer based on the hfs of 11H and 12H as well as the lfs of 10H and 13H; and finally, Peak 3 can be assigned to the 9,11-*cis* isomer, because it exhibits the isomerization-shift characteristics of both the 9-*cis* (the lfs of 8H) and 11-*cis* (the hfs of 11H and 12H and 13H) configurations.

Fig. 3 depicts the NOE correlations which have been used to confirm the above configurational assignments (see the structure of each *cis* isomer): (b) The 7-cis configuration of Peak 4 is confirmed by the NOE correlation between 5Me and 9Me; (c) the 9-cis configuration of Peak 2 is confirmed by the NOE correlation between 8H and 11H; (d) the 11-cis configuration of Peak 1 is confirmed by the NOE correlation between 10H and 13H; and (e) the 9,11-di-cis configuration of Peak 3 is confirmed by the NOE correlations between 8H and 11H as well as between 10H and 13H. The s-cis configuration around the 6C-7C axis is proved for all the isomers by the NOE correlations between 1Me and 7H as well as between 5Me and 8H (5Me and 9Me in the case of the 7-cis isomer). The rest of the NOE correlations in each cis isomer establishes the configuration of its all-trans part(s).

Table 3 (upper part) lists the vicinal coupling constants for the olefinic Hs. All of the vicinal coupling constants through the conjugated single bonds are 12 Hz. Concerning the vicinal coupling constants through the *trans* double bonds, those of 7H=8H are 16 Hz and those of 11H=12H are  $14 \sim 15$  Hz; this difference indicates increased conjugation in the central part. The vicinal coupling constants for the unmethylated-*cis* double bonds, i.e. those of the 7H=8H bond in the 7-*cis* isomer and of the 11H=12H bond in both the 11-*cis* and the 9,11-*cis* isomers, are all 12 Hz. These values approximately agree with those of 7-*cis* [10] and 11-*cis*-retinal [18]; and 7-*cis*- $\beta$ carotene [12]. All of the above values of coupling

	Peak 6 (all-trans)	Peak 4 (7-cis)	Peak 2 (9-cis)	Peak 1 (11-cis)	Peak 3 (9,11-cis)
7H	6.33	5.98	6.32	6.34	6.35
		(-0.35)	(-0.01)	(+0.52)	(+0.02)
8H	6.15	6.12	6.67	6.22	6.68
		(-0.03)	(+0.52)	(-0.07)	(+0.53)
10H	6.21	6.26	6.12	6.62	6.53
		(+0.05)	(-0.09)	(+0.41)	(+0.32)
11H	7.03	6.96	7.12	6.82	6.91
		(-0.07)	(+0.09)	(-0.21)	(-0.12)
12H	6.64	6.61	6.58	6.41	6.36
		(-0.03)	(-0.08)	(-0.23)	(-0.28)
13H	6.95	6.94	6.93	7.39	7.39
		(-0.01)	(-0.02)	(+0.44)	(+0.44)
15H	9.43	9.42	9.41	9.49	9.50
		(-0.01)	(-0.02)	(+0.06)	(+0.07)

Chemical shifts (isomerization shifts<sup>a</sup>) of the olenic H signals of isomeric  $C_{20'}$  aldehyde in chloroform-d<sub>1</sub> (ppm)

<sup>a</sup> Isomerization shifts larger than 0.08 are underlined.

constants support the set of assignments of the H signals and of the *cis-trans* configurations.

## 3.2. Isolation by HPLC and configurational determination by <sup>1</sup>H-NMR spectroscopy of the cis-trans isomers of $C_{25'}$ aldehyde

Fig. 2(b) shows the HPLC elution profile for the *cis-trans* isomers of  $C_{25'}$  aldehyde. Peaks 1, 2, 3, 4, 5, 6 and 8 will be assigned to the 13,13'-cis, 13'-cis, 9,13'-cis, 13-cis, 9,13-cis, 9-cis and all-trans isomers (vide infra). <sup>1</sup>H-NMR spectroscopy of Peak 7 showed that it is a degradation product. The order of elution concerning the mono-cis and all-trans isomers is: 13'-cis, 13-cis, 9-cis and alltrans. In C<sub>25</sub> aldehyde [15], it was 15-cis, 13-cis, 9-cis, all-trans and then 13'-cis. The position of the terminal-methyl group drastically affects both the kind of cis isomers produced (vide infra) and the order of elution (see Fig. 1 for the chemical structures of these aldehydes). Concerning the order of elution, the general trend that an isomer having a *cis* bend on the carbonyl side elutes faster than an isomer having it on the  $\beta$ -ionone side (vide supra) holds in the above pair of aldehydes except for the case of the 13'-cis isomer. Namely, the order of elution is 13-cis, 9-cis and all-trans for C<sub>25'</sub> aldehyde, whereas it is 15-cis, 13-cis, 9-cis and all-trans for C<sub>25</sub> aldehyde. The unique CH=C(CH<sub>3</sub>)-CH=O structure of  $C_{25}$  aldehyde (instead of the C(CH<sub>3</sub>)=CH-CH=O structure of  $C_{25'}$  aldehyde) may enhance the intermolecular interaction between 13'-cis  $C_{25}$  aldehyde (see Fig. 3(e) of [15] for its structure) and the flat surface of silica-gel, and give rise to a longer retention time.

Table 4 lists the chemical shifts of the olefinic Hs and the isomerization shifts (in parentheses) for isomeric C<sub>25'</sub> aldehyde. Here also, COSY correlations were used to establish the assignments of the H signals (data not shown). The following configurational assignments of the cis isomers have been made on the basis of the isomerization shifts: Peak 6 can be assigned to the 9-cis isomer on the basis of the hfs of 10H as well as the lfs of 8H and 11H; Peak 4 can be assigned to the 13-cis isomer based on the hfs of 14H and the lfs of 12H and 15H; and Peak 2 can be assigned to the 13'-cis isomer based on the hfs of 13'H and the lfs of 12'H and 15'H. Concerning the di-cis isomers, Peak 5 can be assigned to the 9,13-cis isomer, because it exhibits the isomerization shifts characteristic of both the 9-cis configuration (the hfs of 10H and the lfs of 8H and 11H) and the 13-cis configuration (the hfs of 14H and the lfs of 12H and 15H). Peak 3 can be assigned to the 9,13'-cis isomer; it shows the isomerization-shift characteristics of both the 9-cis configuration (the hfs of

Table 2



Fig. 3. The configurations of a set of cis-trans isomers of  $C_{20'}$  aldehyde determined in the present investigation: (a) all-trans (Peak 6), (b) 7-cis (Peak 4), (c) 9-cis (Peak 2), (d) 11-cis (Peak 1), and (e) 9,11-cis (Peak 3). NOE correlations which were identified in the NOESY spectra are indicated by arrows.

10H and the lfs of 8H and 11H) and the 13'-*cis* configuration (the hfs of 13'H and 15H, and the lfs of 12'H and 15'H). Peak 1 can be assigned to the 13,13'-*cis* isomer, because it exhibits the characteristics of both the 13-*cis* configuration (the hfs of 14H and the lfs of 12H and 15H) and the 13'-*cis* configuration (the hfs of 12'H and 15'H).

Fig. 4 depicts the NOE correlations which have been used to confirm the above configurational assignments: (a) The all-*trans* configuration of Peak 8 is confirmed by the NOE correlations between each methyl group attached to the conjugated chain and the neighbouring olefinic Hs on both sides of the conjugated chain as well as those between a pair of gem-type olefinic Hs. (c) The 13-*cis* and (d) the 13'-*cis* configurations of Peaks 4 and 2 are confirmed by the NOE correlations between 12H and 15H and between 15'H and 12'H, respectively. (e) The 13-*cis* configuration in the 9,13-*cis* isomer of Peak 5 is confirmed by the NOE correlation between 12H and 15H; (f) the 9,13'-*cis* configuration of Peak 3 is confirmed by the NOE correlations between 8H and 11H as well as between 15'H and 12'H; and (g) the 13,13'cis configuration of Peak 1 is confirmed by NOE correlations between 12H and 15H as well as between 15'H and 12'H. (The NOE correlation between 8H and 11H to confirm the 9-cis configuration could be identified in neither the 9-cis nor the 9,13-cis isomer (Fig. 4(b) and (e)): these correlation peaks are supposed to appear in the diagonal region in the 2D spectra.) The s-cis configuration around the 6C-7C axis for all the cis-trans isomers is confirmed by the NOE correlation between 1Me and 7H as well as between 5Me and 8H. The rest of the NOE correlations shown in the figure confirm the configurations of the all-trans parts of each cis isomer.

Table 3 (the lower part) lists a set of vicinal coupling constants obtained for isomeric  $C_{25'}$  aldehyde. The coupling constants of 11H=12H (15 Hz) are smaller than those of 7H=8H (16 Hz), indicating increased conjugation in the central part of the conjugated chain. Those values indicate also that no unmethylated-*cis* configurations

C <sub>20'</sub> aldehyde	Peak 6 (all-trans)	Peak 4 (7-cis)	Peak 2 (11-cis)	Peak 1 (11-cis)	Peak 3 (9,11-cis)		
H8=H	16	12	16	16	16		
0H-11H	12	12	12	12	12		
1H=12H	14	15	14	12	12		
2H-13H	12	12	12	12	12		
25' aldehyde	Peak 8 (all-trans)	Peak 6 (9-cis)	Peak 4 (13-cis)	Peak 2 (13'-cis)	Peak 5 (9,13-cis)	Peak 3 (9,13'-cis)	Peak 1 (13,13'-cis)
H=8H	15	16	16	16	16	16	16
0H-11H	12	11	11	12	12	11	12
1H=12H	15	15	15	15	15	15	15
4H-15H	12	12	11	12	12	12	11
3'H-12'H	12	11	12	12	12	12	11

are present in the set of *cis* isomers. The coupling constants through the single bonds are in the range of 11-12 Hz.

#### 3.3. Effects of the terminal-methyl position and the length of the conjugated chain on the photostationary-state isomeric composition

Table 5 (above the broken line) compares the amounts of the all-trans and mono-cis isomers in the photostationary-state mixtures of C<sub>20</sub>, C<sub>20'</sub>, C222, C25' and C25 aldehydes, which were obtained by direct photo-isomerization of the all-trans isomer in acetonitrile. Comparison of the chemical structures of these aldehydes in Fig. 1 shows that  $C_{20}$  and  $C_{25'}$  aldehydes ( $C_{20'}$  and  $C_{25}$  aldehydes) can be classified into the same group, because the former aldehydes (the latter aldehydes) contain the parallel-methyl (antiparallel-methyl) fragment on the carbonyl end. The difference in the position of the terminal-methyl group, causing these two different type of fragments, does affect the kind of mono-cis isomers produced (see Table 5):  $C_{20}$  aldehyde produces the 7-cis, 9-cis, 11-cis and 13-cis isomers, whereas  $C_{20'}$  aldehyde does not produce the 13-cis isomer and produces only the 7-cis, 9-cis and 11-cis isomers. Concerning the longer pair of aldehydes, C<sub>25'</sub> aldehyde produces the 9-cis, 13-cis and 13'-cis isomers, whereas C<sub>25</sub> aldehyde produces the 9-cis, 13-cis and 15-cis isomers; the 13'-cis isomer in the former is replaced by the 15-cis isomer in the latter. (Note that I<sub>2</sub>-sensitized photoisomerization of C<sub>25</sub> aldehyde additionally produces the 13'-cis isomer; see below the broken line of the table.)

Fig. 5 depicts the compositions of the all-*trans* and mono-*cis* isomers of  $C_{20}$ ,  $C_{20'}$ ,  $C_{25'}$  and  $C_{25}$  aldehydes for the photostationary-state mixtures in acetonitrile. If we consider the above-mentioned grouping of the aldehydes concerning the fragments adjacent to the carbonyl group, we notice the following systematic changes in composition on going from the former group (i.e.  $C_{20'}$  and  $C_{25'}$  aldehydes) to the latter group (i.e.  $C_{20'}$  and  $C_{25'}$  aldehydes). Namely, (1) increase in the amount of the 9-*cis* isomer; (2) increase in the amount of the 11-*cis* isomer; and (3) disappearance of the 13-*cis* isomer on going from  $C_{20}$  to

Table 3

	Peak 8 (all- trans)	Peak 6 (9- <i>cis</i> )	Peak 4 (13-cis)	Peak 2 (13'-cis)	Peak 5 (9,13- cis)	Peak 3 (9,13'- cis)	Peak 1 (13,13' cis)
7H	6.37	6.40	6.39	6.40	6.41	6.40	6.40
		(+0.03)	(+0.02)	(+0.03)	(+0.04)	(+0.03)	(+0.03)
8H	6.42	7.08	6.39	6.42	7.06	7.08	6.40
		(+0.66)	(-0.03)		(+0.64)	(+0.66)	(-0.02)
10H	6.32	6.18	6.26	6.34	6.13	6.19	6.26
		(-0.14)	(-0.06)	(+0.02)	(-0.19)	(-0.13)	(-0.06)
11H	6.82	7.09	6.81	6.83	7.09	7.10	6.82
		(+0.27)	(-0.01)	(+0.01)	(+0.27)	(+0.28)	
12H	6.37	6.33	6.91	6.37	6.87	6.35	6.91
		(-0.04)	(+0.54)		(+0.50)	(-0.02)	(+0.54)
12′H	10.03	10.02	10.02	10.17	10.02	10.15	10.17
		(-0.01)	(-0.01)	(+0.14)	(-0.01)	(+0.12)	(+0.14)
13'H	6.01	5.99	6.00	5.78	5.99	5.76	5.77
		(-0.02)	(-0.01)	(-0.23)	(-0.02)	(-0.25)	(-0.24)
14H	6.03	6.03	5.86	6.06	5.82	6.06	5.88
			(-0.17)	(+0.03)	(-0.21)	(+0.03)	(-0.15)
15H	6.78	6.74	7.60	6.70	7.05	6.65	6.99
		(-0.04)	(+0.82)	(-0.08)	(+0.27)	(-0.13)	(+0.21)
15′H	6.03	6.01	5.97	7.10	5.94	7.07	7.01
		(-0.02)	(-0.06)	$(\pm 1.07)$	(-0.09)	$(\pm 1.04)$	$(\pm 0.98)$

Table 4 Chemical shifts (isomerization shifts<sup>a</sup>) of the olefinic H signals of isomeric  $C_{25^{\circ}}$  aldehhyde in benzene-d<sub>6</sub> (ppm)

<sup>a</sup> Isomerization shifts larger than 0.09 are underlined.

 $C_{20'}$  aldehyde correspond nicely to (1') increase in the amount of the 13-*cis* isomer, (2') appearance of (or in other words, increase in the amount of) the 15-*cis* isomer, and (3') disappearance of the 13'-*cis* isomer on going from  $C_{25'}$  to  $C_{25}$  aldehyde. These systematic changes indicate that the shift of the terminal-methyl group closer to the carbonyl end (changing the parallel-methyl fragment into the antiparallel-methyl fragment) stabilizes the *cis* configuration around 'double bonds a and b' in the resultant antiparallel-methyl fragment (see Fig. 1(h)); on the other hand, it destabilizes the *cis* configuration around 'double bond c' in the particular fragment.

Fig. 1 summarizes all the *cis* configurations so far detected; the results of the present investigation concerning  $C_{20'}$  and  $C_{25'}$  aldehydes are added to those described in the Introduction section. It was known that  $C_{20}$  and  $C_{22}$  aldehydes produce the 11-*cis* isomer, but  $C_{25}$  and  $C_{30}$  aldehyde do not. Present investigation has shown that  $C_{20'}$ aldehyde produces the 11-*cis* isomer, but  $C_{25'}$ aldehyde does not. Now it is clear that the position of the terminal-methyl group is not relevant to the presence or absence of the 11-*cis* isomer; the key factor in the generation of the 11-*cis* configuration (in a parallel-methyl fragment) must be the length of the conjugated chain.

#### 3.4. A possible explanation of the effects of the terminal-methyl position on the photostationary-state composition in terms of 'enhanced polarization of double bonds upon excitation'

#### 3.4.1. General consideration

Presence or absence of a *cis* configuration in a photostationary-state isomeric mixture, which is obtained starting from the all-*trans* isomer, can be determined by two factors: (1) whether isomerization from the all-*trans* to the particular *cis* configuration can take place upon photo-excitation; and (2) whether the particular *cis* configuration is stable enough against additional photo- or thermal-isomerization back to the all-*trans* isomer. Concerning factor (1), photo-isomerization can take place via either the singlet- or the triplet



Fig. 4. The configurations of a set of cis-trans isomers of  $C_{25'}$  aldehyde determined in the present investigation: (a) all-trans (Peak 8), (b) 9-cis (Peak 6), (c) 13-cis (Peak 4), (d) 13'-cis (Peak 2), (e) 9,13-cis (Peak 5), (f) 9,13'-cis (Peak 3) and (g) 13,13'-cis (Peak 1). NOE correlation which were identified in the NOESY spectra are shown by arrows.

excited state. However, the triplet-state isomerization is unlikely under the present experimental conditions (the solutions were not degassed and no triplet sensitizers were added): actually, tripletsensitized photo-isomerization of C15, C17, C20 and C<sub>22</sub> aldehydes produced only small amount of cis isomers in the photo-stationary state, because the cis-to-trans isomerization is extremely efficient and the *trans*-to-cis isomerization is inefficient [19]. The triplet-state isomerization has been ascribed to the formation of 'the triplet-excited region' located in the central part of the conjugated chain, where drastic decrease in the bond order of the central double bonds takes place [6,20]. Here, in this paper, we focus our attention to isomerization caused by excitation to the singlet-excited state and in particular, to the excited-state potential barrier which the trans-to-cis isomerization process to overcome. (The relative energies in the  $S_1$  potential minima at various *cis* and *trans* positions are another factor to be examined in the

future.) We are going to propose an idea of 'polarization of the conjugated double bonds upon excitation' which originates from the excited-state polarization of the terminal carbonyl group as a factor to reduce the potential barrier around each double bond in the process of photo-isomerization in the singlet-excited state.

### 3.4.2. Comparison of the electronic structure between $C_{20}$ and $C_{20'}$ aldehydes

Fig. 6 compares the electronic-absorption spectra of all-*trans* (a)  $C_{20}$  and (b)  $C_{20'}$  aldehydes. They exhibit the same absorption maximum but different profiles.  $C_{20'}$  aldehyde having an antiparallel-methyl fragment shows clearer vibrational structures as  $C_{25}$  aldehyde does [16]. On the other hand, the vibrational structure of  $C_{20}$  aldehyde having a parallel-methyl fragment is not clear as in the case of  $C_{25'}$  aldehyde [16]. Thus, the electronic structures of all-*trans*  $C_{20}$  and  $C_{20'}$  aldehyde

Table 5

Compounds	all-trans	7-cis	9-cis	11-cis	13-cis	15-cis	13'- <i>cis</i>	Others
C <sub>20</sub> aldehyde	44.2	1.4	18.1	16.8	12.2			7.3
•	(40.3)	(1.3)	(19.3)	(26.0)	(13.1)			(-)
C <sub>20'</sub> aldehyde	40.2	5.2	29.3	23.0	n.d. <sup>b</sup>			2.3
20 2	(33.8)	(4.4)	(28.9)	(32.9)				(-)
C <sub>22</sub> aldehyde	40.0	0.1	6.7	14.9	37.2			1.1
22 0	(33.4)	(0.1)	(6.6)	(21.6)	(38.3)			(-)
C <sub>25'</sub> aldehyde	38.1	n.d.	9.5	n.d	34.1	n.d.	5.2	13.1
$C_{25}$ aldehyde	52.6	n.d.	2.8	n.d.	37.2	4.7	n.d.	2.7
C <sub>25'</sub> aldehyde	41.4	n.d.	13.9	n.d.	16.2	n.d.	8.6	19.9
$C_{25}$ aldehyde	56.1	n.d.	12.4	n.d.	11.3	2.1	9.5	8.6

The amounts of mono-*cis* isomers (%) in stationary-state mixtures obtained by photo-isomerization of all-*trans*  $C_{20}$ ,  $C_{20}$ ,  $C_{22}$ ,  $C_{25}$  and  $C_{25}$  aldehydes<sup>a</sup>

<sup>a</sup> Direct reading of the area under peak in the elution profiles detected at 360 nm ( $C_{20}$  and  $C_{20'}$  aldehydes), 400 nm ( $C_{22}$  aldehyde) and 420 nm ( $C_{25}$  and  $C_{25'}$  aldehydes). Corrected values of the fraction of all-*trans* and mono-*cis* isomers, the sum of which are normalized to 100%, are shown in parentheses. Direct photoisomerization in acetonitrite and I<sub>2</sub>-sensitized isomerization in *n*-hexane, are shown above and below the broken line.

<sup>b</sup> n.d. indicates 'not detectable'.

hydes as probed by the  $A_g \rightarrow B_u$  transition are almost the same.

Fig. 7 compares the values of  ${}^{13}$ C (hereafter abbreviated as C) and H chemical shifts between all-*trans* C<sub>20</sub> and C<sub>20'</sub> aldehydes as functions of atomic position. Changes in both the C and H chemical shifts on going from C<sub>20</sub> aldehyde (open circles) to C<sub>20'</sub> aldehyde (closed circles) parallel very closely to those on going from C<sub>25'</sub> aldehyde to C<sub>25</sub> aldehyde (see Fig. 3 [16]). The changes in the latter pair of aldehydes were discussed in detail, in a previous paper [16], in terms of (1) the mesomeric effect in the terminal C=O and the neighbouring C=C bonds, (2) the inductive effect of the terminal methyl group, and (3) the steric effects between the methyl and olefinic Hs. Exactly the same discussion applies to the present case of  $C_{20}$  and  $C_{20'}$  aldehydes; however it will not be repeated here. In the present investigation, we will use each value of C chemical shift as a measure of the electron density on the relevant carbon atom; in other words, we will use a pair of chemical-shift values in a carbon–carbon double bond as a measure of its polarization. The values in Fig. 7(a) lead us to the following conclusions:



Fig. 5. The stationary-state isomeric compositions in the direct photo-isomerization in acetonitrile starting from the all-*trans* isomers of  $C_{20}$ ,  $C_{20}$ ,  $C_{25}$  and  $C_{25}$  aldehydes. Open spaces indicate the rest of the isomers.



Fig. 6. The electronic absorption spectra of (a)  $C_{20}$  and (b)  $C_{20}$  aldehydes.



Fig. 7. The positional dependence of the (a) C and (b) H chemical shifts in  $C_{20}$  and  $C_{20'}$  aldehydes. The chemical shifts which are unique to  $C_{20}$  and  $C_{20'}$  aldehydes are shown in open and closed circles, respectively; those which are in agreement between the two aldehydes are shown in shadowed circles.

(a) the polarization in the 13C=14C bond is much larger in  $C_{20}$  aldehyde than in  $C_{20'}$  aldehyde, because the difference in the values of C chemical shift between 13C and 14C is larger in the former than in the latter. Similarly (see Fig. 3(a) of ref. [16]), the polarization in the 14'C=13'C bond is much larger in  $C_{25'}$  aldehyde than in  $C_{25}$  aldehyde. Further, the directions of the above polarizations are in accord with the polarization anticipated for the terminal carbonyl group  $(C=O \rightarrow C^+ - O^-)$ , i.e. the 15C=O bond in  $C_{20}$  and  $C_{20'}$  aldehydes and the 12'C=O bond in  $C_{25'}$  and  $C_{25}$  aldehydes. (b) The polarization of the 11C=12C bond in  $C_{20'}$ aldehyde is in the same direction as that of the 15C=O bond, whereas the polarization of the 11C=12C bond in  $C_{20}$  aldehyde is in the opposite direction. Similarly [16], the polarization of the 15C=15'C bond in C<sub>25'</sub> (C<sub>25</sub>) aldehyde is in the same (opposite) direction in relation to the polarization of the terminal 12'C=O bond. These characteristics in the double-bond polarization, i.e. (a) and (b), will be related to the amounts of isomers which are produced by direct photo-excitation of the all-*trans* isomer.

#### 3.4.3. Polarization of conjugated double bonds as a possible factor determining the barrier to photo-isomerization from the trans to the cis configuration

Ponder and Mathies [21] experimentally evidenced large increase in the dipole moment and the polarizability of all-*trans*  $C_{20}$  aldehyde (retinal) upon excitation to the B<sub>u</sub> state. The dipole moment increased by  $13.2 \pm 0.6$  D upon excitation, and the solution-phase dipole moment in the excited state was estimated to be  $19.8 \pm 0.7$  D. On the other hand, increase in the long-axis polarizability upon excitation was 600 + 100 Å<sup>3</sup>. The results suggest that strong polarization can take place upon excitation of the present aldehydes to the  $B_{\mu}$  state, especially in a polar solvent. On the other hand, the generation of the 11-cis isomer by direct photo-excitation of the all-trans isomer takes place not in a non-polar solvent, *n*-hexane, but in a polar solvent, acetonitrile [10,11]. This observation strongly supports the idea that the polarization of the conjugated chain must play a key role in isomerization *via* the singlet-excited state. Here, we propose a hypothetical mechanism of photo-isomerization, which includes (i) strong polarization of the carbonyl group upon excitation in the conjugated double bonds, and (ii) its propagation to the neighbouring conjugated double bonds toward the  $\beta$ -ionone ring.

Now, we try to explain, in terms of this polarization mechanism, the effect of the terminalmethyl position on the photostationary-state isomeric composition. Here, (1) we correlate both the magnitude and the direction of polarization in a conjugated double bond (in the ground state) to the magnitude of its enhanced polarization upon excitation, and then, (2) we correlate this excitedstate polarization to the amounts of *cis* isomers produced. Explanations are as follows (see Figs. 1, 5 and 7(a)): (a) the polarization of the 13C=14Cbond, which is parallel to that of the 15C=O bond, is increased on going from  $C_{20'}$  to  $C_{20}$ aldehyde (vide supra). An assumption that this trend is enhanced upon singlet excitation can explain the fact that the 13-cis isomer can be produced not in  $C_{20'}$  aldehyde but in  $C_{20}$  aldehyde, because stronger polarization must be more advantageous for the *trans* to *cis* isomerization. Similarly, the polarization of the 14'C=13'C bond is increased on going from  $C_{25}$  to  $C_{25^{\prime}}$  aldehyde (vide supra), a fact which explains that the 13'-cis isomer can be produced not in  $C_{25}$  aldehyde but in  $C_{25'}$  aldehyde. (2) The parallel polarization of the 11C=12C bond in reference to the terminal C=O bond in the case of C<sub>20'</sub> aldehyde (in contrast to the antiparallel polarization in C<sub>20</sub> aldehyde)

explains that the larger amount of the 11-cis isomer can be produced in  $C_{20'}$  aldehyde than in  $C_{20}$  aldehyde. Similarly, the parallel (antiparallel) polarization of the 15C=15'C bond in C<sub>25</sub> aldehyde ( $C_{25'}$  aldehyde) explains the presence (absence) of the 15-cis isomer in  $C_{25}$  aldehyde ( $C_{25'}$ aldehyde). (3) The polarization of the 9C=10C bond are almost the same in both  $C_{20}$  and  $C_{20'}$ aldehyde in the ground state. However, the direction of polarization in the neighboring 11C=12C bond may affect a cooperative enhancement of polarization upon excitation in the conjugated double bonds. The 9C=10C and 11C=12C parallel (antiparallel) polarization in  $C_{20}$  aldehyde ( $C_{20}$ aldehyde) may enhance (suppress) the excitedstate polarization, and as a result, it produces a larger (smaller) amount of the 9-cis isomer in  $C_{20'}$ aldehyde (C<sub>20</sub> aldehyde). Similarly, the parallel (antiparallel) polarization in  $C_{25}$  aldehyde ( $C_{25'}$ aldehyde) should increase (decrease) the amount of the 13-cis isomer produced. Thus, the effect of the terminal-methyl position on the stationarystate composition can be qualitatively explained in terms of the enhancement of the ground-state polarization upon excitation. However, theoretical calculations concerning the singlet-excited state and more quantitative experimental evidence are necessary to establish this hypothetical mechanism.

#### 4. Conclusion

Direct photo-isomerization of all-*trans*  $C_{20'}$  aldehyde in acetonitrile produced the 7-*cis*, 9-*cis*, 11-*cis* and 9,11-*cis* isomers, whereas I<sub>2</sub>-sensetized photo- isomerization of all-*trans*  $C_{25'}$  aldehyde in *n*-hexane produced the 9-*cis*, 13-*cis*, 13'-*cis*, 9,13-*cis*, 9,13'-*cis* and 13,13'-*cis* isomers. (In the latter aldehyde, direct photo-isomerization in acetoni-trile produced the same set of isomers.)

In order to determine the effects of the terminal-methyl position on the types and the amounts of *cis* isomers which are generated by direct photo-isomerization of the all-*trans* isomer in acetonitrile, the compositions of mono-*cis* isomers were compared between  $C_{20}$  and  $C_{20'}$  aldehydes as well as between  $C_{25'}$  and  $C_{25}$  aldehydes. The shift of the particular methyl group closer to the carbonyl end caused the following systematic changes in the isomeric composition: (1) increase in the amount of the 9-*cis* isomer; (2) increase in the amount of the 11-*cis* isomer; and (3) disappearance of the 13-*cis* isomer on going from  $C_{20}$ to  $C_{20'}$  aldehyde correspond, on the basis of the structural similarity in the carbonyl side of the conjugated chain, to (1') increase in the amount of the 13-*cis* isomer, (2') appearance of the 15-*cis* isomer, and (3') disappearance of the 13'-*cis* isomer on going from  $C_{25'}$  to  $C_{25}$  aldehyde. These changes can be explained in terms of enhancement of the ground-state polarization upon excitation.

The position of the terminal-methyl group is not relevant to the presence or absence of the 11-*cis* isomer in the set of aldehydes examined. The key factor in the generation of the 11-*cis* configuration (in a parallel-methyl fragment) must be the length of the conjugated chain.

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