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Isolation by high-pressure liquid chromatography of cis–trans isomers of β-apo-12′-carotenal and determination of their configurations by ^{1}H NMR spectroscopy

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Abstract

High-pressure liquid chromatography of an isomeric mixture of β-apo-12′-carotenal, which was obtained by iodine-sensitized photo-isomerization, resolved eleven peaks of cis–trans isomers. The configurations of eight isomers, i.e., all-trans, 9-, 13-, 15-, 13′-mono-cis, and 9,13-, 9,13′- and 13,13′-di-cis, were determined by ^{1}H NMR spectroscopy. ^{1}H,^{1}H COSY and long-range ^{1}H,^{1}H COSY spectra was used for the assignments of all the ^{1}H signals. The isomerization shifts of the olefinic ^{1}H signals and the NOE correlations, which were identified in the ^{1}H,^{1}H NOESY spectra, were used for the configurational determinations. In relation to the difference in isomeric composition between retinoids and carotenoids, the cis configurations found in the present compound (C_{25} aldehyde) are compared with those found in retinal (C_{20} aldehyde) and β-apo-8′-carotenal (C_{30} aldehyde) having a shorter and a longer conjugated chain, respectively.

1. Introduction

Particular cis isomers and cis-to-trans isomerization reactions of retinoids and carotenoids play important roles in photobiology. In retinal proteins, a retinal molecule is bound to the apo-protein as a protonated Schiff base, and its isomerization triggers a series of their physiological reactions. The 11-cis to all-trans isomerization takes place in rhodopsin [1,2], the all-trans to 11-cis isomerization takes place in retinochrome [3], and the all-trans to 13-cis isomerization takes place in bacteriorhodopsin [4–6]. Thus, the presence of the 11-cis isomer in retinal, to bind to the apo-protein of rhodopsin and to be released from retinochrome, is crucial for these proteins to carry out the function of vision. Efficient 11-cis to all-trans and all-trans to 11-cis isomerizations have been shown in a model protonated Schiff base to retinal [7].

In the reaction center and the light-harvesting complexes of purple photosynthetic bacteria, the natural selection of the carotenoid configurations has been found [8]. In the

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light-harvesting complex, the stable all-trans isomer is selectively bound, and its unique internal conversion characteristics among the low-lying singlet states are supposed to facilitate two channels of singlet energy transfer from the carotenoid Bu^+ and $2Ag^-$ states to the bacteriochlorophyll Q_x and Q_y states, respectively [9]. In the reaction center, on the other hand, the 15-cis isomer is non-covalently bound, and the 15-cis carotenoid is expected to play a most important role in the photo-protective function, i.e., quenching triplet bacteriochlorophyll to avoid sensitized generation of harmful singlet oxygen. Extremely efficient isomerization from 15-cis to all-trans in the triplet state was found for a carotenoid [10–13], and a molecular mechanism of dissipation of the triplet energy by the carotenoid, which includes isomerization around the C15=C15′ bond, has been proposed [13,14]. Thus the presence of the 15-cis carotenoid in the reaction center is crucial for the photo-protective function.

It is intriguing to address questions such as why the 11-cis configuration, which plays physiologically an important role in retinal, has not been found in carotenoids, and why the 15-cis configuration, instead, is present. Both the 11-cis and the 15-cis configurations are characterized by efficient isomerization into the all-trans configuration, and these particular isomerizations are used for the physiological functions as described above. Fig. 1 compares a set of all-trans-aldehydes having different lengths of the conjugated chain, i.e., (a) C_{20} aldehyde (retinal), (b) C_{22} aldehyde (retinylideneacetaldehyde) and (c) C_{30} aldehyde (β-apo-8′-carotenal). Arrows indicate the double bonds at which the cis configurations have been identified. The 11-cis configuration is found in C_{20} and C_{22} aldehydes [16,17], but it is not found in C_{30} aldehyde [15]. Zechmeister [18] proposed the idea that the cis configuration of the $C(CH_3)$=CH–CH=CH–$C(CH_3)$ partial structure is sterically prohibited. However, this type of cis configuration has been actually found as the 7-cis isomers of retinal [19] and of β-carotene [20]. Therefore, the steric hindrance is probably not the reason for the absence of the 11-cis configuration in β-apo-8′-carotenal and in all the other naturally-occurring carotenoids.

A possible hypothesis is that the absence of the 11-cis configuration is due to the entire length of the conjugated chain, which can affect the electronic states of the partial structure; we first examined this possibility. In the present investigation, we have selected C_{25} aldehyde (β-apo-12′-carotenal in Fig. 1(d)) as a key compound having a conjugated chain whose length is in-between those of the retinoids and the carotenoid. We have determined the configuration of each cis isomer in an isomeric mixture which was obtained by iodine-sensitized photo-isomerization. In this compound, the 15-cis and 13-cis isomers were identified by ^{1}H-NMR spectroscopy [21], but no systematic search for the 11-cis isomer has been attempted.

2. Experimental

2.1. Isolation of isomeric β-apo-12′-carotenal

A set of cis–trans isomers of β-apo-12′-carotenal were collected by means of high-pressure liquid chromatography from an isomeric mixture, which was obtained by iodine-sensitized photo-isomerization of the all-trans isomer. A *n*-hexane solution containing 1.75×10^{-5} to 6×10^{-5} M all-trans-β-apo-12′-carotenal (a gift from Dr. Alexander Angerhofer) and 2.5×10^{-7} to 2.0×10^{-6} M iodine was irradiated for 0.5–4 h with a fluorescence lamp (25 W) from a distance of 10–25 cm. The HPLC conditions were as follows: column, 4 mm i.d. × 300 mm for analysis and 8 mm i.d. × 250 mm for sample collection; adsorbate, Daisogel Si sp-60, 5 μm; eluent, 7% diethyl ether in *n*-hexane; flow rate, 1.0–1.2 ml min^{-1}; and detection, 450 nm. The purity of the isomer which was used for each NMR measurement was estimated by assuming the same ε value of the set of isomers as follows: peak 1, 100%; peak 2, 95%; peak 4, 85%; peak 6, 93%; peak 7, 97%; peak 8, 93%; peak 10, 100% and peak 11, 100%.

2.2. NMR measurements

The 1D ^{1}H NMR spectrum and the ^{1}H,^{1}H COSY, long-range COSY, and NOESY spectra of each isomer (0.2–3.5 mg) were recorded at 8 °C in benzene-d_6 (CEA 99.6%) by the use of JEOL JNM-GX-400 or a JNM-GX-500 NMR spectrometer. Pulse sequence of each measurement was as follows. ^{1}H,^{1}H COSY, $90°-t_1-90°-t_2$; long-range ^{1}H,^{1}H COSY, $90°-t_2-90°-\Delta-t_2$ with $\Delta = 400$ ms; and ^{1}H,^{1}H NOESY, $90°-t_1-90°-\tau_m-90°-t_2$ with $\tau_m = 600$ ms. The resolution of each 1D spectrum was 0.24 Hz.

3. Results and discussion

3.1. Elution profile of the isomeric mixture

Fig. 2 shows the HPLC elution profile of an isomeric mixture which was obtained by the iodine-sensitized photoisomerization of the all-trans isomer in *n*-hexane. Silica-gel adsorption chromatography using a 4 mm i.d. × 300 mm column and an eluent, 7% diethyl ether in *n*-hexane, resolved the mixture into eleven peaks of isomers. The all-trans isomer turned out to be the major component, peak 10. Eight components, i.e., peaks 1, 2, 4, 6, 7, 8, 10 and 11, were isolated by preparative HPLC; correspondence of each isolated component to the numbered peak in the mixture was confirmed by co-chromatography. ^{1}H NMR spectroscopy of those isomers lead us to the following configurational assignments (vide infra): peak 1, 15-cis; peak 2, 13-cis; peak 4, 13,13′-cis; peak 6, 9,13-cis; peak 7, 9-cis; peak 8, 9,13′-cis; peak 10, all-trans; and peak 11, 13′-cis. The following trends are seen in the order of elution for the set of isomers in relation to their configurations (see Fig. 3 for the configurations of the isomers).

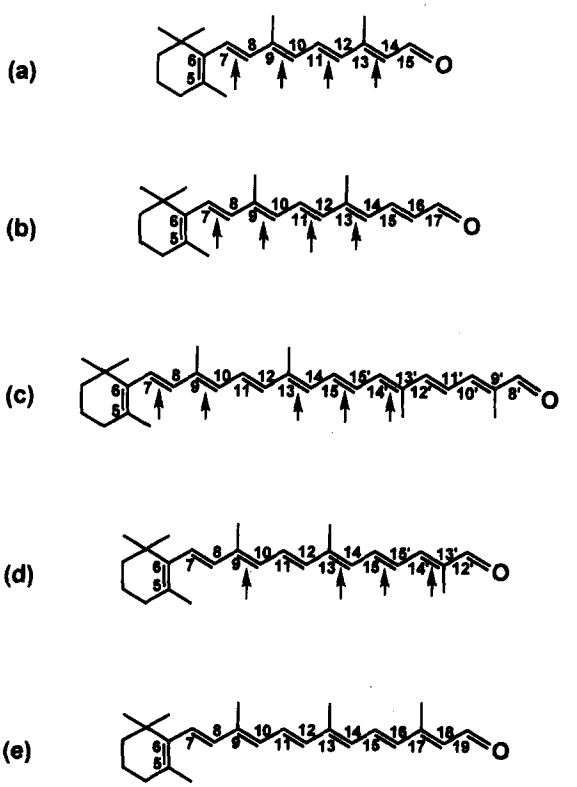


Fig. 1. The structures of all-trans aldehydes having a conjugated chain with different lengths and with different positions of the methyl groups attached to it. (a) Retinal (C_{20} aldehyde), (b) retinylideneacetaldehyde (C_{22} aldehyde), (c) β-apo-8′-carotenal (C_{30} aldehyde), (d) β-apo-12′-carotenal (C_{25} aldehyde) and (e) 13-(2,6,6-Trimethyl-1-cyclohexenyl)-3,7,11-trimethyl-2,4,6,8,10,12-tridecahexenal (C_{25} aldehyde).

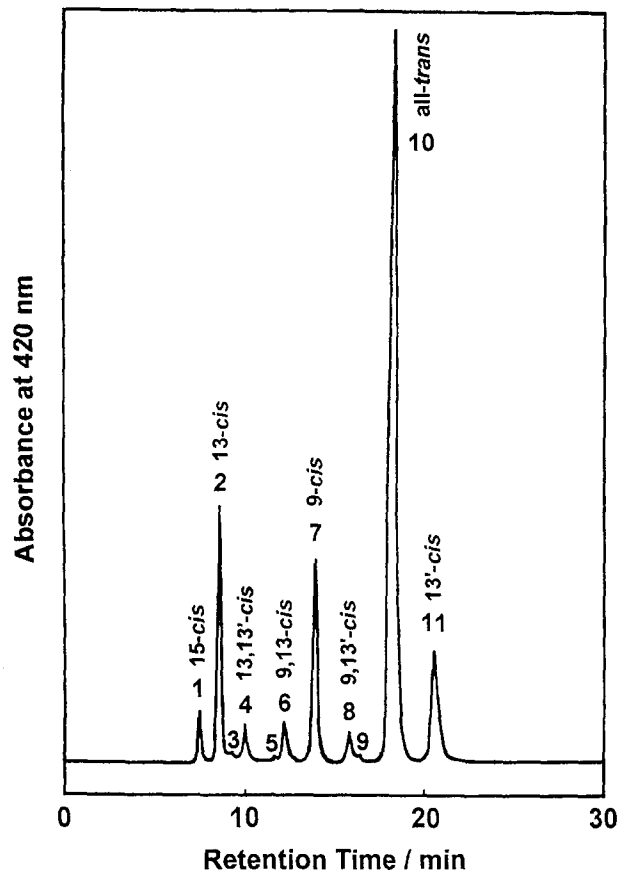


Fig. 2. The elution profile of an isomeric mixture which was obtained by I_2-sensitized photoisomerization of all-trans-β-apo-12'-carotenal in n-hexane. Peak numbers and the results of configurational determinations are indicated.

(1) A central-mono-cis isomer tends to elute faster than a peripheral-mono-cis or the all-trans isomer. The retention times are in the order, 15-cis, 13-cis < 9-cis < all-trans, 13'-cis, although the order between 15-cis and 13-cis as well as that between all-trans and 13'-cis are reversed.

(2) A di-cis isomer tends to elute between the corresponding mono-cis isomers. The retention times are in the order, 13-cis < 9,13-cis < 9-cis; and 9-cis < 9,13'-cis < 13'-cis; and 13-cis < 13,13'-cis < 13'-cis.

(3) The 13'-cis configuration seems to have stronger affinity to the adsorbate than the all-trans configuration, although both all-trans and 13'-cis have similar stretched configurations. The retention times are in the order, all-trans < 13'-cis; 9-cis < 9,13'-cis; and 13-cis < 13,13'-cis.

3.2. Assignment of the ^1H signals and configurational determination for the all-trans isomer

Fig. 3(a) shows the configuration of all-trans-β-apo-12'-carotenal and the numbering of the carbon atoms in the backbone, including the β-ionone ring and the conjugated chain; hereafter, the ^{1}H's attached to the backbone directly (^{1}H will be abbreviated as H), or the Hs in the methyl group attached to it, will be named by the use of the numbers, e.g., 8H or 9-methyl H. Table 1 lists all the H–H correlations which were detected in the COSY, long-range COSY and NOESY spectra. The COSY peaks correlate the olefinic Hs between a pair of tertiary (or between a quaternary and a tertiary) carbon atoms, while the long-range COSY peaks correlate a methyl H with the adjacent pair of olefinic Hs. Starting from the 9.47 ppm signal of 12'H attached to the carbonyl group, the above

correlations lead us to an unequivocal set of assignments of all the H signals. Table 2 lists the values of chemcial shift for the olefinic Hs. The chemical shift values for the rest of Hs are as follows: 1-methyl, 1.16 ppm; 5-methyl, 1.82 ppm; 9-methyl, 1.93 ppm; 13-methyl, 1.78 ppm; 13′-methyl, 1.83 ppm; 2-methylene, 1.51 ppm; 3-methylene, 1.60 ppm; and 4-methylene, 1.98 ppm. Table 1 lists also the H–H NOE correlations found in the NOESY spectrum. A part of the correlations, which are necessary to establish the backbone configuration of the all-trans isomer, are depicted in Fig. 3(a). The NOE correlation between 1-methyl H and 7H and that between 5-methyl H and 8H indicates the s-cis configuration around the 6–7 single bond. The NOE correlation between 14′H and 12′H as well as that between 15′H and 13′-methyl H establish the trans configuration around the 13′=14′ double bond. The rest of the NOE correlations (see for details the arrows in Fig. 3(a)) guarantee the all-trans configuration in the central part of the conjugated chain. Thus, the assignments of all the H signals as well as the all-trans backbone configuration are now established for the all-trans isomer.

3.3. Configurational assignments of the cis isomers based on isomerization shifts

A set of assignments of the H signals was obtained for each isomer on the basis of the COSY and long-range COSY correlations, as in the case of the all-trans isomer. Table 2 lists the values of the chemical shifts and the 'isomerization shifts' (shown in parentheses) of the olefinic H signals for the set of isomers. Table 3 lists a set of vicinal coupling constants of the olefinic Hs in all the cis–trans isomers; the values support our assignments of the olefinic H signals. The 'isomerization shifts' are defined as changes in the chemical shifts for a cis isomer with reference of those for the all-trans isomer. When a cis-bend is introduced, high-field-shifts (hfs) take place in the convex side and low-field-shifts (lfs) take place in the concave side due to changes in the H–H steric interaction, a rule which has been widely used for configurational determinations of varioius carotenoids [22,23]. Application of this rule leads us to the following set of configurational assignments (see Figs. 3(b)–(h) and the underlined isomerization shifts in Table 2, most of which are characteristics of each cis configuration). Peak 7 shows the hfs

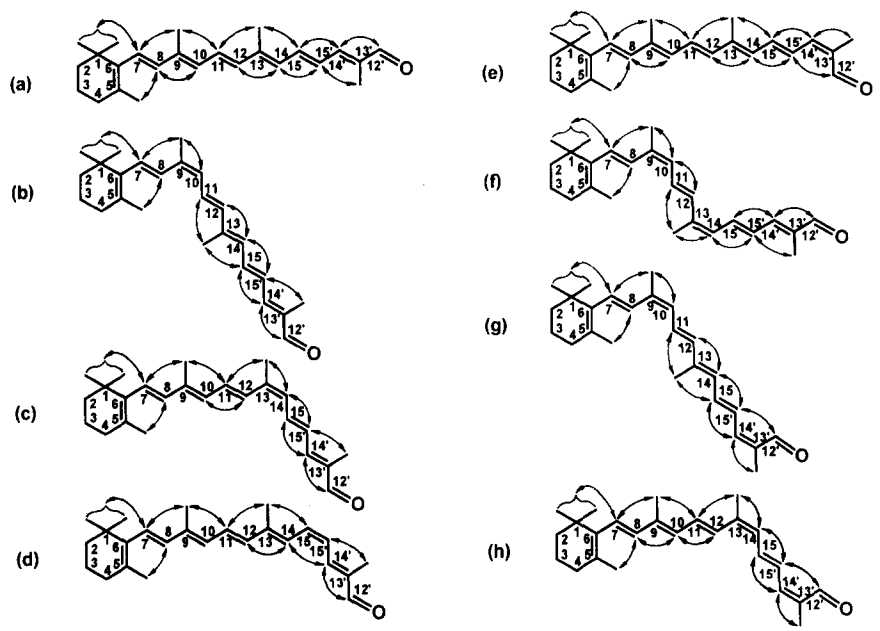
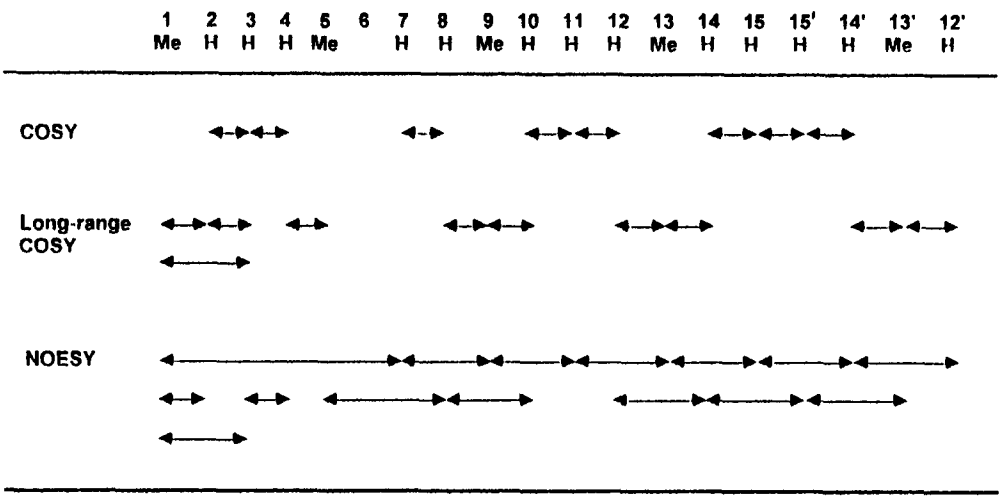


Fig. 3. The configurations of a set of cis–trans isomers of β-apo-12′-carotenal (C_{25} aldehyde) determined in the present investigation. NOE correlations which were identified in the NOESY spectra and facilitated the configurational determinations are shown with arrows. (a) Peak 10, all-trans; (b) peak 7, 9-cis; (c) peak 2, 13-cis; (d) peak 1, 15-cis; (e) peak 11, 13′-cis; (f) peak 6, 9,13-cis; (g) peak 8, 9,13′-cis and (h) peak 4 13,13′-cis.

Table 1
H–H correlations detected in two-dimensional spectra



of the 10H signal and the lfs of both the 8H and the 11H signals, and no such large isomerization shifts are seen for the rest of the olefinic Hs. Therefore, it can be assigned to the 9-cis isomer. Peak 2 shows the hfs of the 14H signal and the lfs of the 12H and 15H signals; thus, it can be assigned to the 13-cis isomer. Peak 1 shows the hfs of the 15H and 15'H signals as well as the lfs of the 14H and 14'H signals, and thus, it is to be assigned to the 15-cis isomer. No large isomerization shifts are seen in the rest of the

Table 2
Chemical shifts (isomerization shifts[a]) of the olefinic H signals of isomeric β-apo-12'-carotenal in benzene-d_6 (in ppm)

	Peak 10 (all-trans)	Peak 7 (9-cis)	Peak 2 (13 -cis)	Peak 1 (15-cis)	Peak 11 (13'-cis)	Peak 6 (9,13-cis)	Peak 8 (9,13'-cis)	Peak 4 (13,13'-cis)
7H	6.38	6.41 (+0.03)	6.40 (+0.02)	6.38	6.36 (−0.02)	6.43 (+0.05)	6.37 (−0.01)	6.37 (−0.01)
8H	6.42	7.10 (+0.68)	6.47 (+0.05)	6.42	6.52 (+0.10)	7.08 (+0.66)	7.19 (+0.77)	6.57 (+0.15)
10H	6.33	6.17 (−0.16)	6.41 (+0.07)	6.38 (+0.05)	6.32 (−0.01)	6.26 (−0.07)	6.18 (−0.15)	6.39 (+0.06)
11H	6.85	7.11 (+0.26)	6.85	6.86 (+0.01)	6.85	7.11 (+0.26)	7.09 (+0.24)	6.83 (−0.02)
12H	6.40	6.34 (−0.06)	6.92 (+0.52)	6.41 (+0.01)	6.40	6.87 (+0.47)	6.35 (−0.05)	6.91 (+0.51)
12'H	9.47	9.47	9.47	9.42 (−0.05)	9.47	9.48 (+0.01)	9.47	9.47
14H	6.07	6.06 (−0.01)	5.88 (−0.19)	6.56 (+0.49)	6.09 (+0.02)	5.83 (−0.24)	6.07	5.88 (−0.19)
14'H	6.41	6.35 (−0.06)	6.43 (+0.02)	7.02 (+0.61)	6.38 (−0.03)	6.43 (+0.02)	6.36 (−0.05)	6.30 (−0.11)
15H	6.63	6.56 (−0.07)	6.85 (+0.22)	6.47 (−0.16)	6.63	6.83 (+0.20)	6.58 (−0.05)	6.86 (+0.23)
15'H	6.36	6.35 (−0.01)	6.29 (−0.07)	6.13 (−0.23)	6.41 (+0.05)	6.26 (−0.10)	6.37 (+0.01)	6.45 (+0.09)

[a] Isomerization shifts larger than 0.10 are underlined.

Table 3
Vicinal coupling constants[a] of the olefinic H signals of isomeric β-apo-12′-carotenal in benzene-d_6 (in Hz)

	Peak 10 (all-trans)	Peak 7 (9-cis)	Peak 2 (13-cis)	Peak 1 (15-cis)	Peak 11 (13′-cis)	Peak 6 (9,13-cis)	Peak 8 (9,13′-cis)	Peak 4 (13,13′-cis)
7H=8H	15	16	16	16	16	16	16	16
10H–11H	12	11	11	12	12	11	12	11
11H=12H	15	15	15	15	15	15	15	15
14H–15H	12	12	11	12	12	12	11	11
14′H–15′H	12	11	12	12	12	12	11	12

[a] The 15=15′H coupling constants could not be determined due to possible long-range couplings.

parts of these two isomers, a fact which indicates that they are mono-cis isomers. Peak 6 exhibits isomerization shifts which are characteristic of both the 9-cis and the 13-cis configurations, i.e., the hfs of the 10H signal and the lfs of the 8H and 11H signals as well as the hfs of the 14H signal and the lfs of the 12H and 15H signals. Therefore, it can be assigned to the 9,13-di-cis isomer. Peak 11 shows no clear set of isomerization shifts ascribable to any particular cis-bend, but the chemical shifts are definitely different from those of the all-trans isomer. Therefore, it may be assigned to the 13′-cis isomer; the 'isomerization shifts' for this type of peripheral 13′-cis configuration has not been established. Peak 8 shows a set of isomerization shifts to be ascribed to the 9-cis configuration, i.e., the hfs of the 10H signal and the lfs of the 8H and 11H signals. Some changes in chemical shifts are seen in the rest of the part, when compared to the chemical shifts of the 9-cis isomer. Therefore, it may be assigned to the 9,13′-di-cis isomer. Peak 4 shows the isomerization shifts characteristic of the 13-cis configuration, i.e., the hfs of the 14H signal and the lfs of the 12H and 15H signals, and the chemical shifts of the rest of the part are different from those of the 13-cis isomer. Therefore, it may be assigned to the 13,13′-di-cis isomer. The NOE correlations establish the configurational assignments of these three isomers having the 13′-cis configuration, which will be described in the next section.

3.4. Configurational assignments of the cis isomers based on the NOE correlations

Figs. 3(b)–(h) depict two types of NOE correlations for the cis isomers, one between a pair of olefinic Hs and the other between a methyl H and an olefinic H, which have played definitive roles in determining the cis–trans configuration around each carbon–carbon, double or single bond. Correlations between a pair of olefinic Hs which give rise to signals in a similar magnetic field could not be identified, because their NOE peaks appear in the diagonal region of the NOESY spectra. However, the observed correlations were enough to establish the configuration of each isomer as follows.

The NOE correlation between 1 methyl H and 7H as well as that between 5-methyl H and 8H prove the s-cis configuration around the 6–7 bond for all the cis isomers. The NOE correlation between 9-methyl H and 10H, that between 13-methyl H and 14H, and that between 14H and 14′H, in peak 7, peak 2 and peak 1, establish the 9-cis, 13-cis and 15-cis configurations, respectively. The NOE correlations between 14′H and 12′H and that between 15′H and 13′-methyl H in all the above isomers indicate the trans configuration around the terminal 13′=14′ bond. The NOE correlations for the rest of the parts of the isomers guarantee an all-trans configuration. Therefore, peaks 7, 2 and 1 can be definitely assigned to the 9-, 13- and 15-mono-cis isomers, respectively.

The NOE correlation between 9-methyl H and 10H and that between 13-methyl H and 14H in peak 6 establish the presence of both the 9-cis and 13-cis configurations in its structure. The NOE correlation between 14′H and 12′H as well as that between 15′H and 13′-methyl H shows the '13′-trans' configuration as in the above three isomers. Thus, peak 6 can be assigned to the 9,13-di-cis isomer. (The rest of the NOE correlations exclude the possibility of a tri-cis configuration.)

The NOE correlation between 14′ and 13′-methyl H as well as that between 15′H and 12′H, which are commonly found in peak 11, peak 8 and peak 4, evidences the terminal 13′-cis configuration in their structures. The NOE correlations in the rest of the part of peak 11 indicate an all-trans configuration, and therefore, peak 11 can be definitely assigned to the 13′-mono-cis isomer. The NOE correlation between 9-methyl H and 10H and that between 13-methyl and 14H which are found in peak 8 and peak 4, respectively, prove the 9-cis and the 13-cis configurations in their structures. Therefore, peak 8 and peak 4 can be definitely assigned to the 9,13′- and 13,13′-di-cis isomers, respectively. Thus, NOE correlations have enabled an unequivocal set of configurational assignments for the three isomers having the terminal 13′-cis configuration.

3.5. Cis configurations found in β-apo-12′-carotenal: difference in stable isomers between retinoids and carotenoids

First, we summarize the cis isomers which have been identified in the present investigation: Seven different kind of cis isomers, including 9-cis, 13-cis, 15-cis, 13′-cis, 9,13-cis, 9,13′-cis and 13,13′-cis (see Fig. 3), were identified in the isomerization mixture obtained by iodine-sensitized photo-isomerization of the all-trans isomer in *n*-hexane. Fig. 1(d) depicts the double bonds where the cis configurations have been found; the 9-cis, 13-cis, 15-cis and 13′-cis configurations are found, but the 7-cis and 11-cis configurations are not. The elution profile in Fig. 2 exhibits some very minor components, i.e., peak 3, peak 5 and peak 9, whose configurations have not been determined yet. Therefore, the possibility that one of them is actually the 11-cis isomer cannot be excluded at the present stage. HPLC analysis of an isomeric mixture which was obtained by direct photo-excitation (without sensitizer) in acetonitrile suggested, based on the retention times, the generation of the 15-cis, 13-cis, 9-cis and all-trans isomers and additional three minor components. HPLC analysis of an isomeric mixture obtained by melting the crystalline all-trans isomer (thermal isomerization) showed the generation of the 15-cis, 13-cis, 9-cis and 9,13-cis isomers as well as one medium (peak X) and several minor unidentified components. Raman spectroscopy of peak X showed that it is neither the 11-cis isomer nor the 7-cis isomer, because the key Raman line of the unmethylated-cis configuration [16,17] was not observed. Based on all the above results, it can be safely concluded that 11-cis isomer, if any, is extremely unstable in the present key compound, i.e., C_{25} aldehyde.

Second, we compare the above results of C_{25} aldehydes with those of C_{20} and C_{22} aldehydes having a shorter conjugated chain, and also with those of C_{30} aldehyde having a longer conjugated chain. In C_{20} and C_{22} aldehydes (Fig. 1(a) and (b)), the 7-cis, 9-cis, 11-cis and 13-cis configurations have been identified [16,17]. Typical isomerization mixtures of C_{20} and C_{22} aldehydes, which were obtained by direct photo-excitation in acetonitrile, contained the 11-cis isomer in the amounts relative to that of all-trans, 0.54 and 0.57, respectively, although no 11-cis isomers were generated in *n*-hexane [16,17]. Thus, in those lower aldehydes, the 11-cis isomers were found in considerable amounts in the isomeric mixtures, and they were stable enough to be isolated by HPLC. (They were the least stable isomer once they were excited to the T_1 state [24].)

In C_{30} aldehyde (Fig. 1(c)), the 7-cis, 9-cis, 13-cis, 15-cis and 13′-cis configurations have been found in an isomeric mixture, which was obtained by iodine-sensitized photoisomerization in *n*-hexane. In this higher aldehyde, no 11-cis isomer has been identified either by direct photo-isomerization in acetonitrile or by iodine-sensitized isomerization in *n*-hexane.

Finally, we try to figure out possible reasons why the 11-cis configuration has not been identified in the present key compound (C_{25} aldehyde).

(1) The kind of isomers to be generated can depend on the isomerization conditions. In C_{20} and C_{22} aldehydes, the 11-cis isomer could be generated by direct photo-isomerization in acetonitrile. In C_{25} and C_{30} aldehydes, on the other hand, the 11-cis isomers were not generated even by direct photo-excitation in acetonitrile. Thus, the idea that

the absence of the 11-cis isomer depends on the isomerization conditions can be excluded.

(2) The kind of isomers to be generated can depend on the length of the conjugated chain. This is the idea which motivated the present investigation on C_{25} aldehyde as a key compound. However, it does not seem to be the case.

(3) The following idea can be more likely: the electronic environment of the $C_9(CH_3)$=$C_{10}H$–$C_{11}H$=$C_{12}H$–$C_{13}(CH_3)$ structure, or in other words, a pair of the conjugated groups attached to this structure, i.e., one from the left-hand-side (the β-ionone-side group) and the other from the right-hand-side (the carbonly-side group), can be more important factors to control whether the cis configuration can be generated in the particular structure or not. When we compare the molecular structures of C_{22}, C_{30} and C_{25} aldehydes (Figs. 1(b)–(d)), we notice that the groups attached from the left-hand-side are exactly the same, but that the groups attached from the right-hand-side are different. In particular, the $C_{13}(CH_3)$=$C_{14}H$–$C_{15}H$=$C_{15'}H$–$C_{14'}H$=$C_{13'}(CH_3)$ group which is unique to both C_{25} and C_{30} aldehydes (unique to the carotenoids in general) may prevent the formation of the unmethylated-cis configuration in the neighbouring $C_9(CH_3)$=$C_{10}H$–$C_{11}H$=$C_{12}H$–$C_{13}(CH_3)$ structure through a modification of its electronic environment. From this viewpoint, $C_{25'}$ aldehyde (Fig. 1(e)) must be another key component to be examined in order to test this interpretation. (If this interpretation is correct, the 11-cis configuration must be found.)

4. Conclusions

The present results strongly suggest that the positions of the methyl groups attached to the conjugated backbone, rather than the length of it, should play a most important role in determining the set of isomers to be generated. Final conclusion should wait for the results on the new key compound, $C_{25'}$ aldehyde.

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