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# DETERMINATION OF THE GEOMETRIC CONFIGURATION OF THE POLYENE CHAIN OF MONO-*CIS* $C_{40}$ CAROTENOIDS II

## A $^{13}C$ NMR STUDY OF MONO-*CIS* LUTEINS AND MONO-*CIS* CAPSANTHINS‡

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**Abstract** Systematic chromatography has resulted in the isolation of four geometric isomers as products of iodine-catalysed isomerisation of lutein. $^{13}C$ NMR spectral analysis has established their stereochemistry as mono-*cis* luteins with a *cis*-configured double bond at C-9, C-9', C-13 and C-13', respectively. Carbon-13 NMR has also provided unambiguous stereochemical assignment for the previously isolated four mono-*cis* capsanthins

Lutein ((3*R*,3'*R*,6'*R*)-β, ε-carotene-3,3'-diol) **1**, the yellow pigment of autumn leaves and a great many blossoms, features a constitutionally asymmetric, all-*trans* decaene chromophore and two, structurally and sterically different end groups.[2] According to the pioneering studies of Zechmeister *et al.*,[3,4] the iodine-catalysed isomerisation of **1** yields two isomeric products, designated neoluteins A and B. On the basis of UV/visible spectral data, their respective stereochemistries were tentatively assigned by the same authors as C-13 or C-15 mono-*cis* and C-9 or C-9' mono-*cis* luteins.[5b] Careful and systematic chromatographic studies carried out in this laboratory previously had, however, disclosed[6] that the iodine-catalysed stereomutation of other, constitutionally asymmetric carotenoids gave, by virtue of the non-equivalence of positions C-9 and C-9', C-13 and C-13', four rather than just two geometric isomers. A typical example is provided by capsanthin (**6**), the main pigment of paprika, which resulted in four mono-*cis* isomers.[6] Hindered (C-7, C-7' or C-11, C-11') and central (C-15) mono-*cis* isomers[5a,7] generally claimed not to appear in *trans-cis* equilibria, although a few natural carotenoids are known to have *cis* double bond(s) in these positions.[8-10]

As part of our systematic investigations into the isolation, identification and stereomutation of carotenoids, we now report our chromatographic and $^{13}C$ NMR results for the main isomerisation products of lutein. In our earlier studies,[6] the nature and site of geometric isomerism of four stereomutation products of capsanthin (**6**) were tentatively inferred from the electronic spectra. In view of the uncertainties of this approach, a reinvestigation using the accurate methods of $^{13}C$ NMR spectroscopy seemed to be warranted. The results of these studies are also presented in this communication.

Isomerisation of **1** was carried out by iodine-catalysis in diffuse daylight. Column chromatography of the reaction mixture afforded neoluteins A and B in accord with earlier studies.[3,4] Rechromatography under strictly controlled conditions revealed that, in reality, both of these products constitute a mixture of two separable components named, in the order of their relative adsorption affinities, neoluteins A' (**2**) and A'' (**3**; from neolutein A) and neoluteins B' (**4**) and B'' (**5**; from neolutein B), respectively.

The separated and recrystallised isomers show the same mass fragmentation pattern[11] ($M^+$ at *m/e* 568, and fragment ions at *m/e* 550 (M-18), 476 (M-92), 462 (M-106), 458 (M-110), 430 (M-138), etc.) which, in turn, is identical to that obtained for the parent (**1**). Some differentiation between the isomers is possible on the basis of their electronic spectra. Both **2** and **3** exhibit strong absorption at approx. 330 nm ("*cis*-band"), also noted for neolutein A;[3,4] they do, however, differ in the values of the ratio $Q = E_{max}/E_{cis\text{-band}}$, being 1.94 for **2** and 2.28 for **3**, as well in the magnitudes of the hypsochromic shift of their $\lambda_{max}$ relative to the value in the all-*trans* (**1**). By contrast, the occurrence of a weak *cis*-band is characteristic for both **4** and **5** and no considerable difference in their $\lambda_{max}$ shifts could be observed. The above findings suggest[5c,d] that isomer **2** has its *cis*-configured double bond in a more central (C-13) position of the decaene chromophore than does **3** (C-13'), whilst **4** and **5** are presumably mono-*cis* isomers of **1** with the *cis* double bond in a periferial position, as it had originally been proposed for their mixture, neolutein B.[3,4]

By similar reasoning, analysis of the electronic spectra of neocapsanthins (A' **7**, A'' **8**, B' **9** and B'' **10**), the iodine-catalysed stereomutation products of capsanthin (**6**), suggested[6] that these are mono-*cis*

‡For part I, see Ref. 1.

Table 1. $^{13}C$ chemical shifts[a] of lutein and capsanthin isomers

| Carbon | (1)[b] | (2)[c] | (3)[c] | (4)[b] | (5)[b] | (6)[d] | (7)[d] | (8)[d] | (9)[d] | (10)[d] |
|---|---|---|---|---|---|---|---|---|---|---|
| C-1 | 36.76 | 36.83 | 36.83 | 36.72 | 36.69 | 37.14 | 37.12 | 37.16 | 37.10 | 37.14 |
| C-2 | 48.41 | 48.43 | 48.43 | 48.40 | 48.31 | 48.52 | 48.49 | 48.57 | 48.46 | 48.53 |
| C-3 | 64.34 | 64.55 | 64.55 | 64.24 | 64.28 | 65.07 | 65.02 | 65.10 | 65.03 | 65.05 |
| C-4 | 42.61 | 42.65 | 42.65 | 42.60 | 42.59 | 42.63 | 42.61 | 42.67 | 42.61 | 42.63 |
| C-5 | 126.69 | 126.81 | 126.67 | 126.68 | 126.96 | 126.31 | 126.45 | 126.31 | 126.54 | 126.30 |
| C-6 | 137.26 | 137.31 | 137.31 | 137.22 | 137.45 | 137.79 | 137.71 | 137.83 | 138.06 | 137.80 |
| C-7 | 125.81 | 125.88 | 125.88 | 125.76 | 127.62 | 125.91 | 126.23 | 125.82 | 127.70 | 125.81 |
| C-8 | 137.92 | 137.99 | 137.99 | 137.88 | 130.13 | 138.46 | 138.35 | 138.52 | 130.69 | 138.49 |
| C-9 | 135.48 | 135.80 | 135.98 | 135.41 | 133.99 | 135.91 | 136.00 | 135.96 | 134.68 | 135.94 |
| C-10 | 131.03 | 131.01 | 131.01 | 131.02 | 129.52 | 131.66 | 131.19 | 131.29 | 129.62 | 131.30 |
| C-11 | 124.88 | 124.75 | 125.88 | 124.86 | 123.56 | 125.54 | 126.77 | 125.35 | 124.32 | 125.36 |
| C-12 | 137.27 | 137.34 | 129.11 | 137.22 | 136.56 | 137.42 | 129.21 | 137.50 | 136.68 | 137.49 |
| C-13 | 136.09 | 136.05 | 134.54 | 136.06 | 135.98 | 137.59 | 136.78 | 137.29 | 137.45 | 137.22 |
| C-14 | 132.44 | 132.27 | 130.80 | 132.42 | 132.37 | 132.39 | 130.37 | 132.24 | 132.28 | 132.47 |
| C-15 | 129.99 | 129.48 | 128.69 | 129.96 | 129.90 | 129.73 | 128.85 | 130.65 | 129.79 | 129.87 |
| C-16 | 28.63 | 28.66 | 28.66 | 28.61 | 28.65 | 28.76 | 28.76 | 28.79 | 28.81 | 28.77 |
| C-17 | 30.27 | 30.29 | 30.29 | 30.26 | 30.29 | 30.29 | 30.29 | 30.32 | 30.34 | 30.30 |
| C-18 | 21.55 | 21.57 | 21.57 | 21.53 | 21.63 | 21.62 | 21.61 | 21.62 | 21.74 | 21.62 |
| C-19 | 12.61 | 12.69 | 12.69 | 12.60 | 20.56 | 12.78 | 12.81 | 12.77 | 20.76 | 12.76 |
| C-20 | 12.68 | 12.69 | 20.59 | 12.67 | 12.70 | 12.87 | 20.76 | 12.88 | 12.93 | 12.85 |
| C-1' | 34.00 | 34.06 | 34.06 | 33.90 | 33.98 | 43.98 | 43.97 | 44.01 | 43.99 | 44.02 |
| C-2' | 44.89 | 44.96 | 44.96 | 44.93 | 44.90 | 50.96 | 50.94 | 51.02 | 50.98 | 50.95 |
| C-3' | 63.46 | 63.66 | 63.66 | 63.36 | 63.39 | 70.35 | 70.29 | 70.36 | 70.32 | 70.31 |
| C-4' | 125.97 | 126.18 | 126.18 | 126.12 | 126.02 | 45.39 | 45.36 | 45.44 | 45.41 | 45.42 |
| C-5' | 135.55 | 135.67 | 135.85 | 135.23 | 135.45 | 58.96 | 58.94 | 59.01 | 58.96 | 59.05 |
| C-6' | 54.77 | 54.83 | 54.83 | 54.94 | 54.76 | 202.88 | 202.92 | 202.92 | 202.89 | 203.43 |
| C-7' | 129.13 | 129.11 | 129.11 | 131.29 | 129.12 | 120.97 | 120.94 | 121.37 | 120.98 | 122.35 |
| C-8' | 137.35 | 137.34 | 137.34 | 129.48 | 137.35 | 146.87 | 146.89 | 146.76 | 146.90 | 138.16 |
| C-9' | 134.88 | 135.46 | 134.85 | 133.45 | 134.84 | 133.65 | 133.56 | 134.11 | 133.62 | 131.65 |
| C-10' | 130.52 | 130.52 | 130.52 | 128.97 | 130.50 | 140.67 | 140.69 | 140.50 | 140.70 | 139.21 |
| C-11' | 124.80 | 125.88 | 124.69 | 123.58 | 124.77 | 124.11 | 123.97 | 125.35 | 124.09 | 123.12 |
| C-12' | 137.27 | 129.11 | 137.34 | 136.54 | 137.28 | 141.97 | 142.01 | 133.57 | 142.01 | 140.73 |
| C-13' | 136.14 | 134.59 | 136.27 | 136.00 | 136.01 | 136.12 | 135.73 | 134.37 | 135.83 | 136.12 |
| C-14' | 132.44 | 130.80 | 132.27 | 132.32 | 132.40 | 135.25 | 135.11 | 133.61 | 135.27 | 134.60 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| C-15' | 129.99 | 128.69 | 129.48 | 129.90 | 130.00 | 131.25 | 130.69 | 128.37 | 131.67 | 131.22 |
| C-16' | 23.72 | 23.73 | 23.73 | 23.68 | 23.72 | 25.12 | 25.11 | 25.14 | 25.14 | 25.13 |
| C-17' | 29.56 | 29.58 | 29.58 | 29.55 | 29.56 | 25.91 | 25.91 | 25.94 | 25.93 | 25.91 |
| C-18' | 22.68 | 22.72 | 22.72 | 22.69 | 22.66 | 21.35 | 21.36 | 21.37 | 21.38 | 21.27 |
| C-19' | 12.98 | 13.04 | 13.04 | 20.95 | 12.97 | 12.73 | 12.70 | 12.85 | 12.73 | 20.40 |
| C-20' | 12.68 | 20.59 | 12.69 | 12.76 | 12.67 | 12.83 | 12.81 | 20.53 | 12.83 | 12.94 |

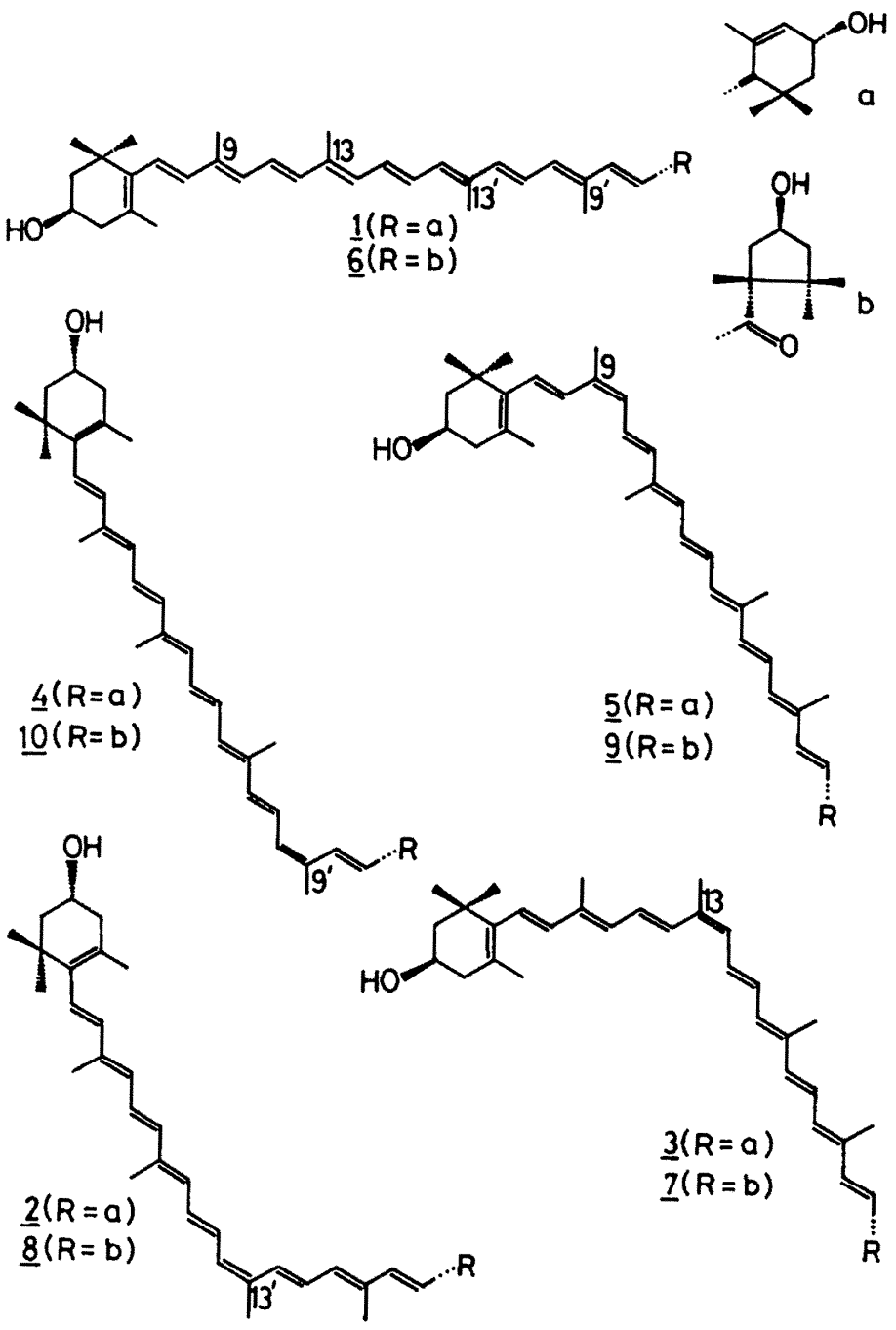
[a] In ppm relative to internal TMS. [b] 18 mg in 0.25 ml $CDCl_3$ + DMSO (2:1) solvent mixture.

[c] 10 mg in 0.20 ml $CDCl_3$ + DMSO (2:1) solvent mixture. [d] 13 mg in 0.20 ml $CDCl_3$.

Table 2. Relevant *trans*-to-*cis* isomerization shifts[a] in lutein and capsanthin

| Carbon | (3) | (5) | (7) | (9) | Carbon | (2) | (4) | (8) | (10) |
|---|---|---|---|---|---|---|---|---|---|
| C-5 | 0.0 | 0.3 | 0.1 | 0.2 | C-5' | 0.1 | -0.3 | 0.1 | 0.1 |
| C-6 | 0.1 | 0.2 | -0.1 | 0.3 | C-6' | 0.1 | 0.2 | 0.0 | 0.6 |
| C-7 | 0.1 | 1.8 | 0.3 | 1.8 | C-7' | 0.0 | 2.2 | 0.4 | 1.4 |
| C-8 | 0.1 | -7.8 | -0.1 | -7.8 | C-8' | 0.0 | -7.9 | -0.1 | -8.7 |
| C-9 | 0.5 | -1.5 | 0.1 | -1.2 | C-9' | 0.6 | -1.4 | 0.5 | -2.0 |
| C-10 | 0.0 | -1.5 | -0.5 | -2.0 | C-10' | 0.0 | -1.6 | -0.2 | -1.5 |
| C-11 | 1.0 | -1.3 | 1.2 | -1.2 | C-11' | 1.1 | -1.2 | 1.2 | -1.0 |
| C-12 | -8.2 | -0.7 | -8.2 | -0.7 | C-12' | -8.2 | -0.7 | -8.4 | -1.2 |
| C-13 | -1.6 | -0.1 | -0.8 | -0.1 | C-13' | -1.6 | -0.1 | -1.8 | 0.0 |
| C-14 | -1.6 | -0.1 | -2.0 | -0.1 | C-14' | -1.6 | -0.1 | -1.6 | -0.7 |
| C-15 | -1.3 | -0.1 | -0.9 | 0.1 | C-15' | -1.3 | -0.1 | -2.9 | 0.0 |
| C-19 | 0.1 | 8.0 | 0.0 | 8.0 | C-19' | 0.1 | 8.0 | 0.1 | 7.7 |
| C-20 | 7.9 | 0.0 | 7.9 | 0.1 | C-20' | 7.9 | 0.1 | 7.7 | 0.1 |

[a] $\Delta = \delta$ cis-isomer $- \delta$ all-trans (Negative sign indicates upfield shift)




derivatives of **6** with the *cis*-configured double bonds, respectively, at C-13', C-13, C-9' and C-9.

The stereochemistry of the isomerisation products, as inferred from the $^{13}C$ NMR spectra, are displayed in **2**–**5** and **7**–**10**. The assigned chemical shifts are summarised in Table 1; the characteristic *trans*-to-*cis* differential shieldings, $\Delta = \delta^{cis\text{-}isomer} - \delta^{all\text{-}trans}$, are presented in Table 2. The assignment or resonances to individual C atoms in the isomers is based on the following arguments: (i) Known assignments for parent carotenoids. For **1**, these are essentia identical to those first published by Moss,[12] except some refinements necessitated by a substantially lar number of resolved resonances in the present wc These refinements were verified in a separate study isomeric deoxy-luteins.[13] Spectral assignments for *trans* (**6**) and its isomerisation products (**7**–**10**) w readily available from our former $^{13}C$ NMR studies

related isomeric capsorubins and zeaxanthins.[1] (ii) Known differential shieldings associated with *trans*-to-*cis* isomerisation in carotenoid systems.[1,12,14] (iii) The consistency of assignments for the given sets of five isomers **1**–**5** and **6**–**10**.

Examination of the data in Table 1 immediately reveals that there is only one in-chain Me resonance per isomerisation product to undergo major displacement upon isomerisation. This finding, together with the values of differential shieldings observed for the in-chain Me C atoms (7.7–8.0 ppm, Table 2) suggests that molecules **2**–**5** and **7**–**10** are in fact mono-*cis* derivatives of the respective parent carotenoids (**1** and **6**) and, furthermore, that the *cis* double bonds are at positions C-13, C-13', C-9 and C-9'.[1,9,12,14,15] Full support for this contention was provided by a detailed spectral comparison which settled the stereochemistries of lutein and capsanthin isomers as displayed in **2**–**5** and **7**–**10**.

It is of certain interest to note that the placements of the *cis*-configured double bonds in the isomeric pairs proved to be reversed (i.e. C-13 vs C-13', C-9 vs C-9' and *vice versa*) with respect to those derived from the electronic spectra. While the available data are insufficient to rationalise the origins of these contradictions they do, however, caution against formal uses of simple rules in interpreting the electronic spectra of carotenoid systems.

## EXPERIMENTAL

*Materials.* Neolutein A' (**2**), A'' (**3**), B' (**4**) and B'' (**5**) were prepared from (3*R*,6'*R*,3'*R*)-lutein (**1**) by iodine catalysis in diffuse daylight. Chromatography on $CaCO_3$ (Biogal, Hungary) with a mixture of benzene and petroleum ether (1:1) gave two main isomers, the so-called "neolutein A" (band 1) and "neolutein B" (band 2).

Rechromatography of band 1 on $CaCO_3$ (Biogal) with a mixture of benzene and petroleum ether (1:1, gradually increased to 3:2) resulted in neolutein A' (**2**) and neolutein A'' (**3**) in a proportion of about 2:3. Distinct separation of band 1 into **2** and **3** was achieved on a semimicroscale.

Rechromatography of band 2 on a mixture of MgO (Biogal)–MgO (VEB Jenapharm)–Celite 545 (Johns-Manville) (4:3:4) with light petroleum (b.p. 50–60°) containing 19% of acetone gave neolutein B' (**4**) and neolutein B'' (**5**) in equal proportions.

Crystallisation of the mixture of neolutein A' (**2**; $\lambda_{max}$ in benzene: 483, 453 and 428 nm) and A'' (**3**); ($\lambda_{max}$ in benzene: 480, 450 and 426 nm) from benzene-light petroleum (b.p. 50–60°) gave a red, amorphous powder, m.p. 87°.

Neolutein B' (**4**) was crystallised from benzene-light petroleum (b.p. 50–60°) as yellow plates, m.p. 130°; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 483 (105.000), 454 (116.000) and 428 (88.600) nm.

Neolutein B'' (**5**) was crystallised in a manner similar to neolutein B'. It gave yellow plates, m.p. 84°; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 483 (98.100), 453 (111.500) and 428 (84.100) nm.

Neocapsanthins A' (**7**), A'' (**8**), B' (**9**) and B'' (**10**) were prepared from (3*R*,3'*S*,5'*R*)-capsanthin (**6**) by iodine catalysis in diffuse daylight. Chromatography on $CaCO_3$ (Biogal) with benzene gave two main isomers, the so-called "neo-capsanthin A" (band 1) and the so-called "neo-capsanthin B" (band 2). Rechromatography of band 1 and band 2 (separately) on $CaCO_3$ (Biogal) with a 400:1 mixture of benzene and ethanol containing 4% water resulted in neocapsanthins A' (**7**), A'' (**8**) in a proportion of 3:2 and neocapsanthins B' (**9**), B'' (**10**) in a proportion of 5:2. **7**, **8**, **9** and **10** were crystallised from benzene-light petroleum (b.p. 50–60°) as amorphous red powder.

Neocapsanthin A' (**7**), m.p. 84°; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 478 (79.200) and 361 (44.400) nm.

Neocapsanthin A'' (**8**), m.p. 82°; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 476 (78.100) and 360 (44.000) nm.

Neocapsanthin B' (**9**), m.p. 87°; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 482 (90.000) nm.

Neocapsanthin B'' (**10**), m.p. 119°; $\lambda_{max}$ ($\varepsilon_{max}$) in benzene: 478 (93.000) nm.

*Instruments.* M.ps were determined on a Boetius hot stage apparatus. UV-visible spectra were recorded on a Perkin Elmer 402 instrument. Mass spectra were measured at 70 eV on a AEI MS-902 apparatus.

The $^{13}C$ NMR spectra were recorded at 25.16 MHz using a Varian XL-100-15 FT NMR instrument with Varian Disk Accessory. Probe temp was maintained at 35°. To take full advantage of spectral comparison between isomerisation products **2**–**5** and **1** as well as between **7**–**10** and **6**, solvent-, concentration- and temp-dependent chemical shift changes were to be kept at a possible minimum, especially for closely spaced resonances. To this end, solute concentrations, solvent composition and probe temp were maintained strictly constant for a given pair of isomers and the appropriate (all-*trans*) reference sample. Uncertainties in the frequency determination were further reduced by using large data tables. (Digital resolution of frequency domain spectra was 0.25 Hz/data point). The accuracy of chemical shift data is within ±0.02 ppm, which is attested by the excellent reproducibility of the shift values for carbon atoms unaffected by stereomutation (Table 1).

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