# 156. Homonuclear *Overhauser* $^1$H-NMR. Experiments on the Carotenoid Pigments Lycopene and Prolycopene

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*Summary*

The recently proposed structure of the carotenoid pigment prolycopene as the 7,9,7′,9′-tetra-*cis* isomer of lycopene has been unambiguously confirmed by a series of homonuclear *Overhauser* $^1$H-NMR. experiments at 270 MHz. Comparative measurements are reported for lycopene.

**Introduction.** – As early as 1941 *Zechmeister et al.* [1] reported the occurrence of prolycopene as the main carotenoid pigment of the ripe tangerine tomato and assumed its structure to be a poly-*cis* stereoisomer of (all-*trans*) lycopene. Based on the spectroscopic techniques available at that time, *Zechmeister* later tentatively proposed a symmetrical penta-*cis* structure in which the central double bond $\Delta^{15}$ and four other sterically unhindered double bonds should be *cis* [2].

Very recently, our extensive study of the $^1$H- and $^{13}$C-NMR. spectra of lycopene and prolycopene [3] led to the proposal of the symmetric 7,9,7′,9′-tetra-*cis* structure for the latter. Since attempts to confirm this structure by a synthesis have not yet been successful [4], we present here further experimental evidence which unambiguously proves the proposed structure.

Interproton nuclear *Overhauser* (NO.) experiments have been shown to be extremely useful for the elucidation of the structure of stereoisomers. The application is based on the known fact [5] that two protons (or groups of protons) A and B which are in close geometric proximity to one another are coupled by the direct dipolar (through space) coupling. If the two types of proton are close enough and hence significantly contribute to the relaxation processes of each other, the saturation of the signal of protons A changes the population of the spin states of protons B (and *vice versa*) causing an enhancement of the signal intensity.

We recently applied this technique for the confirmation of the preferred $\Delta^6$-*trans* structure of carotenoids with retro-end groups such as eschscholtzxanthin, rhodoxanthin, retro-dehydrocarotene and related compounds [6] and for the structural elucidation of some mono-, di- and tri-*cis* isomeric vitamin A analogues [7].

In complex spectra with strongly overlapping signals, as is frequently observed in the case of carotenoids or even more in biomolecules, the usual measurement of an increase of the integral of the signal of a specific proton may be difficult.

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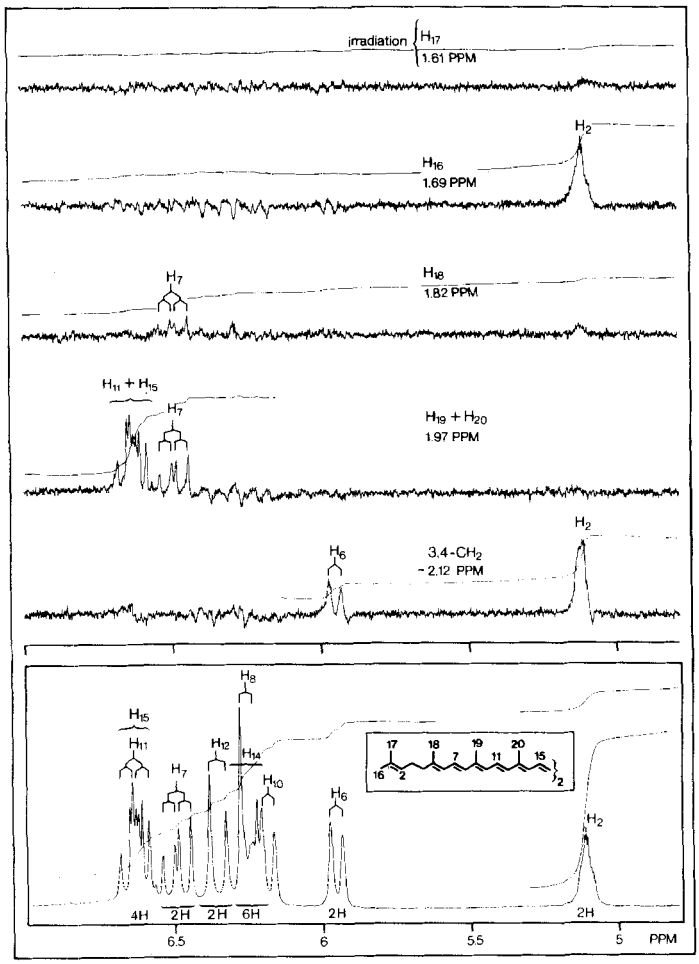


Fig. 1. *NO.-difference spectra and olefinic part of the 270-MHz-$^1$H-NMR. spectrum of lycopene in $CDCl_3$*

However, the direct measurement of the difference free induction decay (FID) with the irradiating radiofrequency alternating on-resonance and off-resonance greatly facilitates the observation of NO.-effects since only those signals appear after *Fourier* transformation in the difference spectrum with positive intensities which are enhanced by the NO.-effect. We utilized this method here for the unambiguous proof of the proposed structure of prolycopene. For comparison, corresponding experiments with the known all-*trans* isomer lycopene are reported.

**Results.** – *Figures 1* and *2* show the olefinic parts (bottom) of the 270-MHz-$^1$H-NMR. spectra of lycopene and prolycopene in $CDCl_3$. The assignment of the different signals was based on various decoupling experiments [3]. It is sufficient to note here that in both cases the two ABC subspectra *(d, d×d, d)* of H–C(6), H–C(7) and H–C(8) and of H–C(10), H–C(11) and H–C(12) were clearly

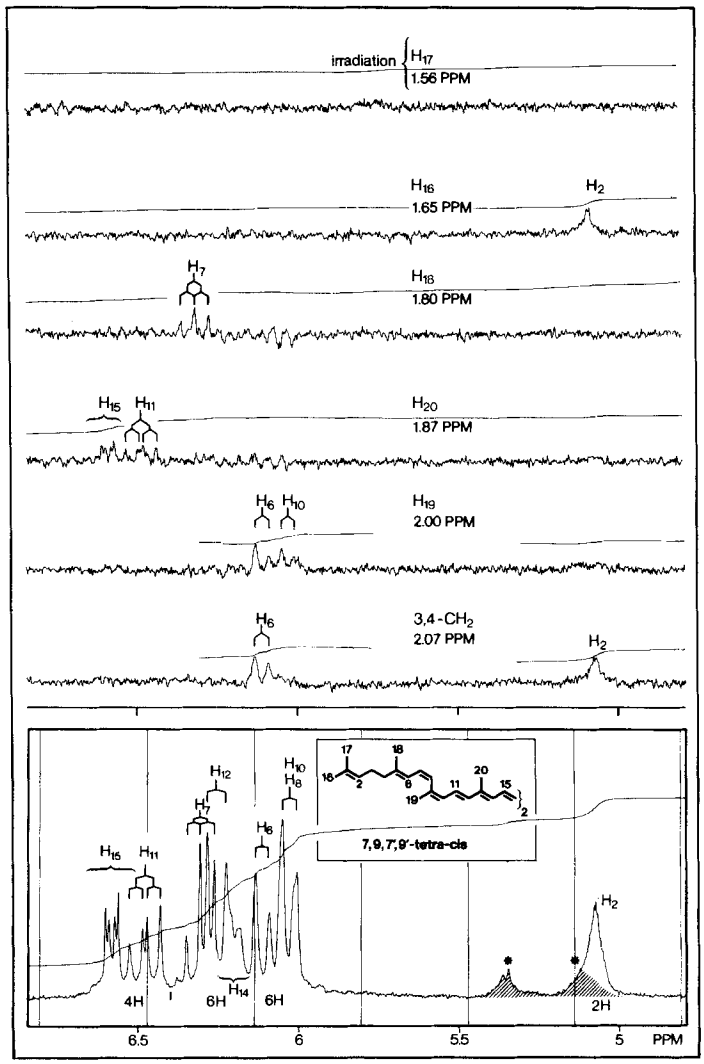


Fig. 2. *NO.-difference spectra and olefinic part of the 270-MHz-$^1$H-NMR. spectrum of prolycopene in $CDCl_3$* (* = impurity signals)

located in this way and the former distinguished from the latter by the small additional couplings of H–C(6) to H–C(4) and H–C(18).

The upper parts of *Figures 1* and *2* show the NO. difference spectra obtained as described in the Experimental Part. Only the signals of those protons are observed with positive intensity which experience an NO.-enhancement upon irradiation of the different aliphatic proton signals as specified in the *Figures*.

In agreement with the known all-*trans* structure of lycopene, *Figure 1*[1]) indicates NO.-enhancements of H–C(7) (approximately 4%) upon irradiation of H–C(18);

[1]) Due to the symmetry of the molecules all statements also relate to protons at primed positions.

of H–C(11), H–C(15) (*ca.* 10% each) and H–C(7) (*ca.* 6%) upon irradiation of the superimposed signals of H–C(19) and H–C(20); and of H–C(6) (*ca.* 6%) and H–C(2) (*ca.* 14%) upon irradiation of the common signal of H–C(3) and H–C(4). In addition, the NO.-effect of H–C(16) on H–C(2) of *ca.* 13% confirmed the known assignment of the former at lower field than H–C(17). It is worth mentioning that the effect of irradiating the signal of H–C(18) on H–C(7) is significantly weaker than upon irradiation of H–C(19).

Corresponding NO.-experiments with prolycopene *(Fig. 2)* revealed that again H–C(7) (NO.-enhancement *ca.* 7%) must be close to H–C(18); H–C(15) and H–C(11) (*ca.* 8% each) close to H–C(20); H–C(6) and presumably H–C(10) (*ca.* 8% each) close to H–C(19); H–C(2) (*ca.* 13%) and more important H–C(6) (*ca.* 10%) must be close to H–C(3) or H–C(4). Together with the observation that the chemical shifts of H–C(14) and H–C(15) of lycopene and prolycopene are very similar and that $\Delta^{15}$ in the latter must, therefore, be *trans* [3] [8], these NO.-experiments unambiguously prove the previously proposed 7,9,7′,9′-tetra-*cis* structures of this poly-*cis* isomer of lycopene.

**Conclusion.** – The experiments discussed here clearly demonstrate that the application of the interproton *Overhauser* effect, if measured by differential gated decoupling, can greatly facilitate the elucidation of the structure of *cis* isomeric carotenoids and related compounds where, in general, a strong overlapping of the different olefinic proton signals is observed.

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**Experimental Part**

The 270-MHz-FT-$^1$H-NMR. spectra were run on a *Bruker* HX-270 spectrometer with ASPECT-2000 computer with 48 K memory (32 K data). The sample concentrations were 3 mg (prolycopene) and 8 mg (lycopene) in 0.5 ml $CDCl_3$ with TMS as internal reference. The sample temperature was 20°. The solutions were degassed by 4 freeze-pump-thaw cycles. The NO.-difference FID's were obtained by gated decoupling (decoupler on for 10 s before every scan) with a microprogram which was virtually identical with the one described in the *Bruker* ASPECT-2000 NMR. Software Manual 1 (part 4). For prolycopene 64 scans (lycopene 80 scans) with irradiation off-resonance were subtracted from those with irradiation on-resonance. A decoupler amplitude of only $\gamma H_2/2\Pi \sim 7$ Hz was utilized in order to minimize the perturbation of other closely situated signals. A flip angle of about 40° was applied. The comparison of the measured integrals with those of the control spectra gave NO.-effects of up to *ca.* 14%.

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