Assignment of the Quaternary Olefinic Carbon Atoms of β-Carotene by 2D ^{1}H,^{13}C-Chemical Shift Correlation *via* Long-range Couplings

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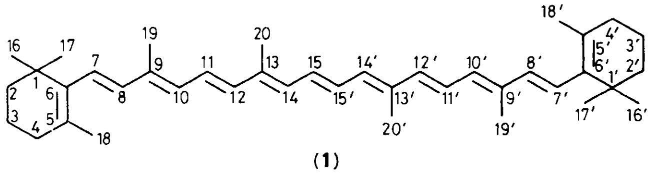
The quaternary carbon atoms of β-carotene can be conveniently assigned by heteronuclear correlation *via* the two- and three-bond couplings with the methyl protons.

The correct assignment of the ^{13}C n.m.r. resonances of the polyene chains of retinoids and carotenoids is a problem of considerable importance in the structural analysis of these molecules and much effort has been made in the last decade to achieve this goal.[1–4] We have recently applied two-dimensional (2D) n.m.r. techniques to polyene systems and have reassigned by ^{1}H,^{13}C-shift correlation spectroscopy[5] the olefinic methine carbon atoms of all-*trans* retinal[6] and β-carotene.[7] In the standard pulse sequence,[5] $90^\circ(^{1}\mathrm{H})\text{-}t_1/2\text{-}180^\circ(^{13}\mathrm{C})\text{-}t_1/2\text{-}\tau_1\text{-}90^\circ(^{1}\mathrm{H},^{13}\mathrm{C})\text{-}\tau_2$-acquire with ^{1}H noise decoupling, the fixed intervals τ_1 and τ_2 were chosen so as to correlate a carbon with its directly attached proton *via* $^{1}J_{\mathrm{CH}}$. However, the problem of assigning the olefinic quaternary carbon atoms still remained, and turned out to be especially difficult in the case of β-carotene, (**1**), because of the lack of a polarizing functional group. Earlier attempts by off-resonance decoupling techniques,[2,3] relaxation time measurements,[8] and chemical shift comparisons in a series of methyl β-apocarotenoates[3] and β-apocarotinals[9] were only partly successful and the conclusions from different research groups have been contradictory until now.[2,3,9]

We demonstrate here that a ^{1}H,^{13}C-shift correlated experiment allows the unambiguous assignment of the olefinic quaternary carbons of (**1**) when the polarization transfer is made *via* the long-range couplings ($^{2}J_{\mathrm{CH}}$, $^{3}J_{\mathrm{CH}}$) with the methyl and methylene protons. Repeated experiments with delays corresponding to different J_{CH} values indicated that maximum polarization transfer was obtained for $J_{\mathrm{CH}} \simeq 10$ Hz. The contour plot of Figure 1 gives the correlations obtained at the chemical shift co-ordinates of the long-range coupled CH pairs. Individual slices at the carbon chemical shift positions (Figure 2) show clearly which protons are involved in the long-range couplings. It can be seen that the methyl protons 3H-C(18) and 3H-C(16,17) correlate with the carbon at δ 138.46. This carbon must be assigned to C(6) since it is the



(**1**)

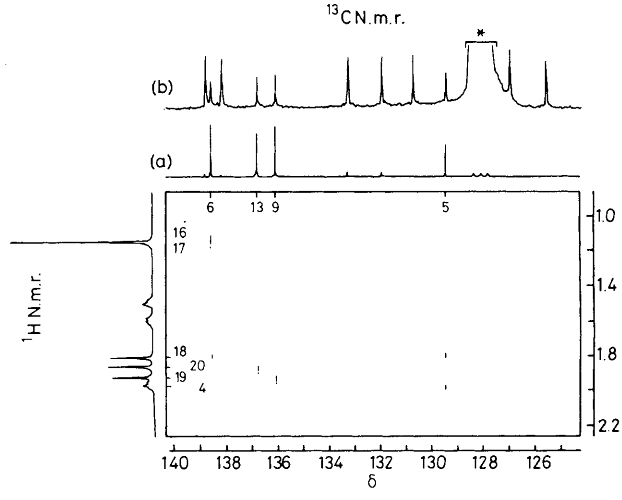


Figure 1. ^{1}H,^{13}C-shift-correlated 2D n.m.r. spectrum at 90.5 MHz of the olefinic carbon region of (**1**) (0.06 M in C_6D_6 at 30 °C). The correlation was made *via* the long-range C,H couplings (τ_1 = 50 ms, τ_2 = 25 ms). The contour plot shows the responses from the aliphatic methyl and methylene protons. Left: 1D ^{1}H n.m.r. spectrum. Top: (a) projection of the 2D data matrix on the ^{13}C chemical shift axis (realise the nulling of the $^{1}J_{\mathrm{CH}}$ correlations); (b) 1D ^{13}C n.m.r. spectrum (the solvent peak is marked by an asterisk).

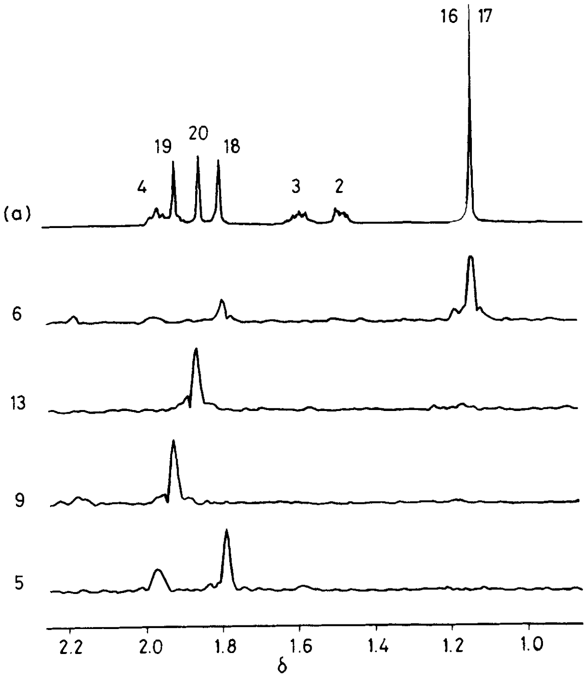


Figure 2. Cross-sections through the ^{13}C chemical shifts in the 2D shift-correlated spectrum of Figure 1. Digital resolution 2 Hz/pt. (a): 1D ^1H n.m.r. spectrum of the aliphatic region of (**1**). The proton assignments are indicated.

only one which can couple *via* $^3J_{CH}$ with the protons of three methyl groups (the assignment is based on the $^4J_{CH}$ couplings being negligibly small).[10] The carbon at δ 129.38 correlates *via* $^2J_{CH}$ with 3H-C(18) and 2H-C(4) and must therefore be attributed to C(5). Finally, it is clearly seen from Figure 2 that the protons 3H-C(19) correlate with C(9) (δ 135.95), while those of 3H-C(20) correlate with C(13) (δ 136.67).

This assignment technique demands that the resonances of the methyl and methylene groups are well-resolved in the one-dimensional ^1H n.m.r. spectrum, a condition which is obtained if (**1**) is dissolved in benzene. The assignment of the methyl protons follows that of the olefinic protons by exploiting the allylic couplings and/or the nuclear Overhauser enhancements.[4]

The size of the coupling constant ($\simeq$ 10 Hz) corresponding to the delays chosen appears overestimated when typical $^2J_{CH}$ and $^3J_{CH}$ couplings involving methyl protons are considered.[10] However the optimum experimental delays are often shorter than those calculated theoretically, a principal reason being that ^1H relaxation during the pulse sequence competes with the detection of the ^{13}C signal.[11]

The analysis of the long-range correlations between the olefinic carbons and the methyl and methylene protons resulted in a much better sensitivity than when the same experiment was performed between the olefinic carbons and the olefinic protons. Obviously, the correlation *via* the methyl protons takes advantage of several favourable circumstances: (a) there is only one pathway for polarization transfer, its sensitivity being increased by the number of equivalent methyl protons; (b) the methyls are expected to give the most intense 2D responses because they appear as singlets in the ^1H n.m.r. spectrum; (c) the similar magnitudes of the two- and three-bond couplings involving the methyl protons allow a simultaneous establishment of connectivities *via* both types of couplings in a single experiment.

From the present experiment we are convinced that 2D $^1H,^{13}C$-shift correlation spectroscopy using the long-range couplings with the methyl protons can be applied as a general tool for the assignment of the quaternary carbons in retinoids and carotenoids, and this should be independent of the number of carbon atoms, if only the ^1H resonances of the various methyl groups can be resolved.

This work was supported by a grant from the Swiss National Science Foundation.

Received, 29th April 1985; Com. 558

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