more basic in the transition state for the mono-hydrogen-bonding catalyzed reaction. This more basic oxygen atom should be stabilized more by an additional acidic hydrogen bond.

In view of the ubiquity of hydrogen bonds and the commonness of polyfunctional catalysis in nature, it seems likely that there are naturally occurring multiple-hydrogen-bonding species that bind substrates and catalyze reactions. Decomposing the epoxides that are formed from carcinogenic polynuclear aromatic compounds before these epoxides react with nucleic acids[4] would be a plausible purpose for either a natural enzyme or a man-made drug.

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Recognition of NMR Proton Spin Systems of Cyclosporin A via Heteronuclear Proton–Carbon Long-Range Couplings

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Homonuclear proton–proton spin coupling is normally used to identify the pattern of spin systems which yields the connectivity of atoms in a molecule (constitution). Recent developments in two-dimensional (2D) correlated NMR techniques (e.g., phase-sensitive H,H-COSY,[1] relayed H,H-COSY,[2] proton double-quantum spectroscopy[3]) provide powerful tools to handle even complicated and crowded spectra. However, extensive overlap of proton signals still often prevents or complicates the detection of proton connectivities.

We demonstrate here that it is possible to spread out proton spin systems by observing them on the normally much more disperse carbon signals. For this purpose the new pulse sequence for heteronuclear correlation via long-range coupling (H,C-COLOC[4]) has been used for aliphatic carbons.

$$90°(^1H),t_1/2,180°(^1H,\ ^{13}C),$$
$$(\Delta_1 - (t_1/2)),90°(^1H,\ ^{13}C),\Delta_2,\mathrm{acq}(^{13}C,\ BB^1H)$$

The H,C-COLOC sequence is especially designed to detect heteronuclear coupling through two and three bonds ($^2J_{CH}$ and $^3J_{CH}$). The experimental details of this sequence are described elsewhere.[4,5]

This sequence has been utilized so far only for quaternary carbon atoms. When the sequence is applied to nonquaternary carbons, the $^1J_{CH}$ couplings evolve during the delay Δ_1 in addition to long-range couplings. This leads to the occurrence of "direct" cross peaks. They can be suppressed by a low-pass J filter.[6] During the delay Δ_2 $^1J_{CH}$ couplings evolve and attenuate the amplitude of the signals from remote C,H-connectivities by a factor $\cos^n \pi J\Delta_2$ for a $^{13}CH_n$ fragment. Therefore Δ_2 has to be chosen in a way that $\cos^n \pi J\Delta_2$ is as near to unity as possible. If the range of $^1J_{CH}$ couplings is small (for peptides $^1J_{CH} = 135 \pm 10$ Hz), such delays Δ_2 are easily found. Nevertheless a selective refocusing of the $^1J_{CH}$ coupling can be achieved with a J-selective π-pulse[7] in the middle of Δ_2.

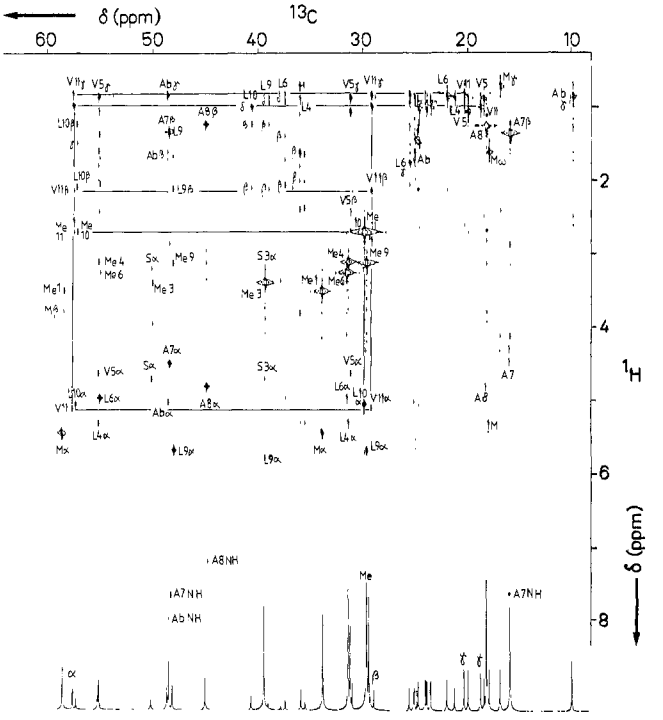

Figure 1. H,C-COLOC spectrum of 500 mg of cyclosporin A in 2.5 mL of $CDCl_3$ and ^{13}C projection of this spectrum (below). 240 increments of 192 scans each, $\Delta_1 = 27$ ms, $\Delta_2 = 37$ ms, duration of one scan 1.25 s, total time 16 h, ^1H frequency 500 MHz. The assignments of most of the cross peaks are given in the contour plot. Cross peaks of MeVal[11] are connected by lines.

However, in our experience the introduction of both spectroscopic tricks did not facilitate the practical evaluation of the spectra.[8] The direct C–H cross peaks provide additional fix-points for the orientation in the spectrum, as they are known from a previously performed H,C-COSY spectrum. The introduction of a J-selective π-pulse yielded additional artifacts due to pulse imperfections.

We demonstrate here the application of the H,C-COLOC experiment to the aliphatic carbon atoms of cyclosporin A (*cyclo*-[-MeBmt[1]-Abu[2]-Sar[3]-MeLeu[4]-Val[5]-MeLeu[6]-Ala[7]-D-Ala[8]-MeLeu[9]-MeLeu[10]-MeVal[11]-])[9] which has a very crowded proton spectrum in the high-field region making the interpretation of the four-Leu systems difficult.[10]

It is possible to solve this problem by the H,C-COLOC experiment which contains carbon *and* proton connectivities of *all* amino acids including their *N*-methyl groups. The contour plot of the H,C-COLOC spectrum of cyclosporin A (Figure 1) exhibits about 120 cross peaks. Carbon signals belonging to the same

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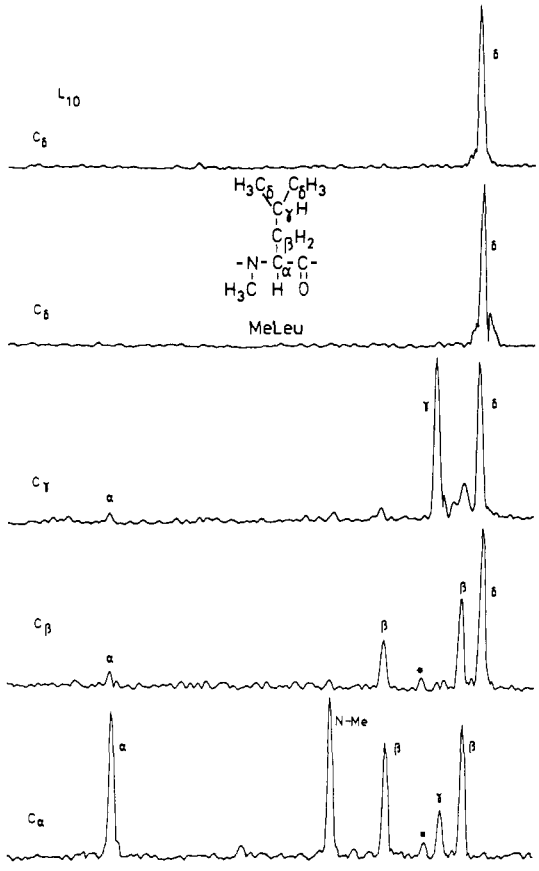

Figure 2. Cross sections through the ^{13}C signals of MeLeu[10] in the plot of Figure 1. The proton assignments are indicated at the signals.

amino acid can be assigned by their coupling to identical protons. For example, the seven *N*-methyl carbons exhibit strong cross peaks to the methyl protons ($^1J_{CH}$) as in a H,C-COSY spectrum. In addition, cross peaks to the α-protons of the corresponding amino acids are observed ($^3J_{CH}$) in the H,C-COLOC spectrum. Similarily, at the α-carbons cross peaks to the α-protons ($^1J_{CH}$) and to the *N*-methyl protons ($^3J_{CH}$) occur. Hence connectivity information of protons and carbons is obtained as indicated in Figure 1 for MeVal[11].

Due to the complexity of the spectrum it is convenient for further interpretation to analyze cross sections through the carbon resonances. As a representative example we show those of the MeLeu[10] residue (Figure 2). As mentioned above, the α-carbon exhibits cross peaks to the *N*-methyl protons and the α-proton. Further signals are found at the position of both β-protons and the γ-proton. This is almost the complete spin system, projected on the α-carbon. The missing δ-protons are already found at the resonance of the β-carbon together with a cross peak to the α-proton and to both β-protons. The γ-carbon shows a weak cross peak to the α-proton in addition to peaks to the γ-proton as well as to the δ-methyl protons. The δ-carbons exhibit cross peaks only to directly attached protons. The coupling pattern of all other amino acids can be traced out in the same way.

Although not all two- and three-bond couplings are detected in the spectrum, it is evident that it contains so much redundant information that in general it appears to be the most informative technique for the determination of proton and carbon connectivities.

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Registry No. Cyclosporin A, 59865-13-3.

An Unusual CIDEP Observation in the Photochemical Reactions of Benzophenone and Ascorbyl Palmitate: The Elusive Neutral Ascorbate Radical

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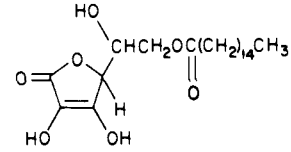
The biochemical and biophysical roles of vitamin C (ascorbic acid) in vivo have received much attention in recent years.[1] In addition to its recognized antioxidant activity[2] this compound has been implicated as a potential therapeutic agent for treatment of ailments ranging from the common cold[3] to cancer.[4] With the establishment of the CIDEP technique as a powerful tool for mechanistic and kinetic studies of photochemical and thermal reactions,[5] the nature of the free radical processes involved in radiolysis[6] and photolysis[7] of vitamin C has been examined and explained by the conventional phototriplet and radical pair mechanisms. However, the relative insolubility of vitamin C has restricted its study to polar solvents.

There is currently considerable interest in the thermal and photochemical reactions of vitamin C derivatives.[8] Ascorbyl palmitate which has utility as an antioxidant in foodstuffs, particularly fats and oils,[9] is of interest as a model for ESR and CIDEP studies; its lipophilic hydrocarbon chain facilitates the study of reactions in nonpolar organic solvents. In this work we report a rather unusual CIDEP observation for the photochemical interaction of ascorbyl palmitate (H_2AP) with several benzophenone derivatives in organic solvents. To our surprise one hyperfine component of the ascorbyl radical spectrum exhibited strong emission while the other component did not show any polarization. This phenomenon can be rationalized by phototriplet CIDEP theory[5] combined with secondary ionization of the elusive neutral HAP radical to the observed radical anion.



CW photolysis of an isopropyl alcohol/toluene (3:7 v/v) solution containing typically 5×10^{-2} M H_2AP and benzophenone, respectively, produced an ESR spectrum as shown in Figure 1a. The central doublet is due to the ascorbate radical anion ($AP^{-}\cdot$) with a *g* factor of 2.0051 and $a_H = 1.69$ G.[10,14] The peripheral weaker lines in Figure 1a can be attributed to the benzophenone ketyl radical ($Ph_2\dot{C}OH$); this same radical could be produced by photolysis of benzophenone in the absence of H_AP.

As shown in Figure 1a the two hyperfine components of $AP^{-}\cdot$ differ dramatically in intensity. Similar results were obtained using 4,4'-dichloro-, 4,4'-dimethyl-, 4,4'-dimethoxybenzophenone with

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