to obtain noticeable concentrations of $Ph_3C^+$.

**Conclusion.** It has been demonstrated that, in a polar solvent such as water, a neutral radical can be photoionized to give the corresponding carbocation. In most cases, the rate of this conversion is likely to be limited only by the length of the ionizing light pulse, in which case it should be possible to produce and study ultrareactive cations with lifetimes, e.g., in the picosecond domain.

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## Proton-Detected 2D Heteronuclear Shift Correlation via Multiple-Quantum Coherences of the Type $I_2S$

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NMR spectroscopy of high molecular weight compounds suffers from overlap problems giving rise to severe difficulties in spectral assignments. 3D NMR measurements[1] are helpful in simplifying spectral patterns but require an enormous amount of computational effort. Alternatively, filtering methods offer the possibility of selectively extracting spectral information from isotope-labeled compounds.[2] In order to provide maximum sensitivity, these so-called editing techniques very often are based on the application of polarization transfer protocols in conjunction with proton detection. We are reporting here on such a pulse sequence, which allows one to exclusively detect heteronuclear spin systems of the type $I_2S$, I and S representing protons and heteronuclei, respectively. This type of selection is extremely useful in discriminating asparagine and glutamine side-chain amide protons from those located in the peptide backbone, as will be shown for uniformly $^{15}N$-labeled RNase $T_1$. Although the $NH_2$ resonances could be identified on the basis of the 0.6 ppm NHD isotope effect, they are likely to be covered by other signals, especially in spectra of larger proteins.

In the following, the basic ideas leading to the design of this particular 2D NMR experiment are summarized. The large heteronuclear one-bond coupling of approximately 90 Hz allows fast excitation of proton-heteronucleus multiple-quantum coherences, including the nitrogen nucleus as a relay site. Usually, the homonuclear coupling constants are 1 order of magnitude smaller and require much longer preparation periods. The selection between different spin topologies depends on these correlations. Consequently, resonances from side-chain amides can be distinguished from those of backbone amides.

A modified DEPT sequence has proven to be suitable for the stepwise preparation of triple-quantum coherences.[3,4] From symmetry considerations, we concluded that a time-reversed DEPT-type sequence would allow the conversion of the triple-quantum coherences into detectable single-quantum terms. This leads to the pulse sequence depicted in Figure 1. In a back-to-back manner, both DEPT pulse clusters flank the evolution period during which chemical-shift labeling of protons with the heteronuclei precession frequencies takes place. Proton chemical-shift contributions were removed by employing selective inversion of proton populations in the middle of the evolution period. Thus, triple-quantum and single-quantum coherences were exchanged, leaving the heteronuclear precession unaffected.

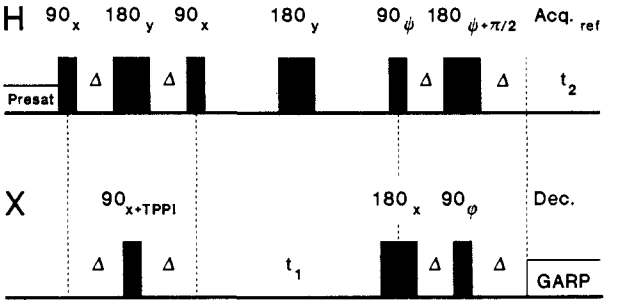


**Figure 1.** Pulse scheme for heteronuclear shift correlation via $I_2S$ three-quantum coherences. The delay Δ should match $1/(2J_{NH})$. The phases are cycled as follows: $\varphi = x, -x$; $\psi = x, x, -x, -x, y, y, -y, -y$; ref = $x, -x, -x, x, -y, y, y, -y$.

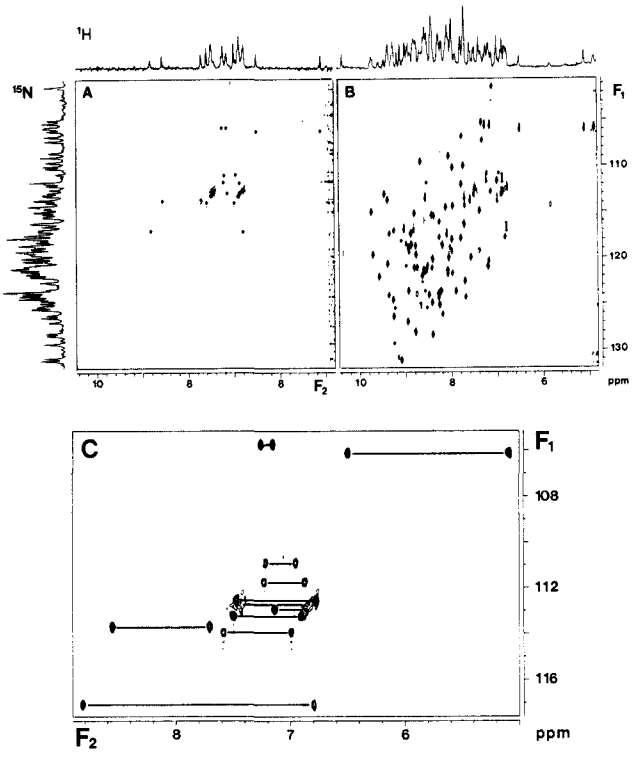


**Figure 2.** Selective heteronuclear shift correlation spectra for terminal amide groups in side chains of asparagine and glutamine residues (A) and for all amides (B) in uniformly $^{15}N$-labeled RNase $T_1$; the $^{15}NH_2$ correlations are indicated in the expansion (C). Both spectra are represented in a pure absorption mode. Prior to FT, the 256 increments of 2K data points were zero-filled once in $t_2$ and twice in $t_1$. The total spectral widths covered 2000 Hz in $F_1$ and 6250 Hz in $F_2$. In order to account for relaxation effects, the delay Δ was set to 5.0 ms, which is slightly shorter than $1/(2J_{NH})$.

Since proton chemical shifts are completely refocused, the experiment reveals pure absorptive signals in the $F_2$ dimension. The signals in $F_1$, owing to delayed $t_1$ acquisition, will be phase-modulated unless an additional refocusing pulse in the nitrogen channel is applied. A refocusing pulse coincident with one of the two proton mixing pulses restores the initial phase relations, thus allowing the nitrogen signals to be represented in a pure absorption mode. Since control of the signal phase is kept separate for each species of nucleus, the TPPI method[5] is applicable for phase-sensitive detection of heteronuclear shifts.

The experiment was performed on a Bruker AM-500 spectrometer equipped with a 5 mm reverse broad-band probe tuned to a 500.13 MHz proton frequency. A sample of fully $^{15}N$-labeled RNase $T_1$ was obtained from *Escherichia coli* by recombinant techniques.[6,7] The isoenzyme contains nine asparagine and two

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glutamine residues. Measurements were carried out with a protein concentration of 3 mM at pH 5.5 and 313 K. During relaxation, the solvent resonance was saturated by low-power excitation. The GARP decoupling scheme[8] was employed during acquisition. Approximately 4 W decoupling power at 50.68 MHz resulted in a $\pi/2$ pulse length of 200 $\mu$s. The total acquisition time for the $^{15}NH_2$-edited spectrum was 6 h, using an extended 64-step phase cycle.

Figure 2A shows the spectral region of the selected amide correlations. A reference spectrum of all backbone and side-chain amide resonances (Figure 2B) was recorded according to the procedures of Bax et al.[9] An expansion (Figure 2C) reveals all 11 of the expected $NH_2$ correlations. In the arginine side chain, the protons undergo rapid exchange at the chosen temperature;[10] therefore, they did not give rise to signals.

Of the few proton resonances in RNase $T_1$ not yet assigned,[11] the asparagine residues in positions 36, 43, 44, 98, and 99 are of particular interest as they are located close to the active site. Their $NH_2$ groups may be used as local spies for the investigation of base recognition and catalysis by means of NMR techniques. The addition of NOE or ROE transfer steps to the pulse sequence could eventually allow tracing of the connectivities to the carbon-bound protons of the amino acid side chains. The usefulness of this method for complete assignment of proton resonances in RNase $T_1$ is currently under investigation in our laboratory.

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## Regioselectivity of the Birch Reduction

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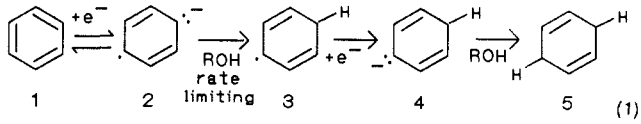
The Birch reduction[1] is one of the most fundamental reactions in organic chemistry. It is known[2] that the reaction proceeds via (a) radical anion formation, (b) protonation by an alcohol, (c) addition of a second electron, and (d) protonation of the resulting cyclohexadienyl carbanion. In 1959 Krapcho and Bothner-By[3a] provided evidence that the rate-limiting step is protonation of the radical anion.[3b] Note eq 1.



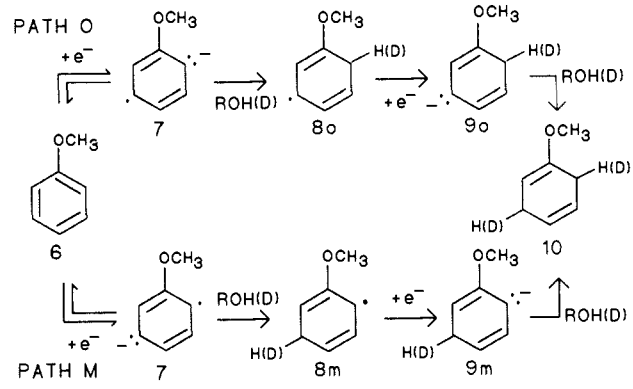

(1)

The regioselectivity follows the Birch rule that the 1,4-dihydrobenzene formed is the isomer having the maximum number of alkoxyl and/or alkyl groups on the residual double bonds. One rationale for this orientation was presented nearly three decades ago.[4] This suggested that there was preferential ortho protonation of the radical anion as the site of the highest electron density, based on Hückel calculations. Anisole is an example that has been the object of particular debate. Whether the initial protonation is ortho or meta has been subject to an unusual variation of viewpoints and speculation as summarized in Table I. In this communication, we report the first experimental test of the initial protonation site in the Birch reduction of anisole.

Table I. Literature Conclusions Regarding Regioselectivity of Protonation of the Radical Anion in the Birch Reduction of Anisole

| suggested preference | basis | ref |
|---|---|---|
| meta | qual mechanistic | 1959[2] |
| meta | qual mechanistic | 1959[3] |
| ortho | Hückel QM | 1961[4] |
| meta | qual mechanistic | 1963[5] |
| none | ESR spin density | 1966[6] |
| meta | SCF QM | 1969[7] |
| ortho | SCF QM | 1980[8] |

Scheme I. Two Alternative Regioselectivities for the Reduction of Anisole




Our research began with and relies on the *premise that the isotope selectivity in a protium–deuterium medium will be greater for a radical anion such as 7 than a carbanion such as* **9**. Since radical anions are known to be considerably less basic and reactive species than carbanions,[9] their protonation consequently will be less exothermic and more selective. Scheme I shows the two potential pathways for the reduction of anisole.

We studied the Birch reduction of anisole in a sodium–liquid ammonia–*tert*-butyl alcohol medium enriched in deuterium. Due to kinetic isotope effects of our original premise, we anticipated that the product site with less deuterium content would be the carbon protonated in the radical anion protonation step. Conversely, the site with greater deuterium should be the one protonated as a nonselective carbanion reacting in a less selective process.

The anisole reduction was run at −78 °C with sodium in liquid ammonia and *tert*-butyl alcohol containing ca. 2% deuterium. The ratio of *m*- to *o*-deuterium content in the 2,5-dihydroanisole product was determined by deuterium NMR analysis utilizing the $Pr(fod)_3$ shift reagent to separate methylene absorption peaks.

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