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Simplification of Complex Proton NMR Spectra

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Abstract: A new NMR pulse sequence is described. It is designed to extract the subspectrum of the proton(s) directly bound to a given carbon atom. The analysis of complicated spectra due to superimposed proton signals is thus greatly simplified. The high-field part of the proton spectrum of strychnine has been investigated by this mean as an example.

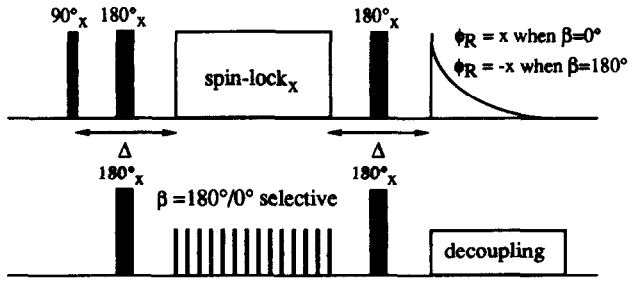
The structural elucidation of organic molecules is nowadays routinely achieved through combined analysis of homo- and heteronuclear 2D NMR spectra. One-dimensional techniques involving the selective excitation of spin systems are intented to yield selected parts of 2D spectra with an improved resolution and shorter recording times[1]. This article describes a method for the selection of proton signals according to the resonance frequency of the attached ^{13}C atom. Proton signals of a given CH_n group can be extracted thus revealing their eventual mutual couplings (if any). Pulse sequences achieving this goal have already been published, but are subject to difficult set-up conditions. The basic problem is the selective excitation of carbon-13 nuclei coupled to protons. This is due to the fact that the direct ^{13}C-^{1}H coupling constants are generally greater than the width of the carbon frequency band to be excited. A first approach by R. Freeman *et coll.*[2] relies on the application of a proton decoupling field simultaneously with the selective carbon nutation period. Sensitivity enhancement and removal of the ^{12}C bound proton signals requires however a preparation period made of nOe transfer and gradient field pulses. During the last years the carbon-proton correlation methods in inverse mode using heteronuclear multiple quantum states filtering[3, 4] have become popular. The 1D adaptation of 2D BIRD-HMQC[5], named SELINCOR[6], uses a rectangular selective read pulse. The resulting sequence hardly combines the necessary selectivity and sensitivity requirements. These drawbacks are partly circumvented by a careful adjustment of a correction delay, as described for the 1D-HMQC-TOCSY[7] experiment.

Our approach is based on the fact that spin-locking the transverse proton magnetisation of an heteronuclear spin system cancels out the action of the heteronucleus-proton coupling interaction. This fact has already been used for the removal of isolated proton signals in HSQC[8] experiments, as a substitute for the lengthy and sometimes poorly efficient BIRD-relaxation procedure[9]. Simultaneous proton spin-locking and selective heteronucleus nutation pulses have proved to constitute an efficient building block for 2D-semiselective heteronuclear correlation experiments[10].

The pulse sequence used (Figure 1) performs a X-filtered[11] proton acquisition in which the Δ defocusing and refocusing periods have been made insensitive to chemical shifts effects by addition of pairs of 180°pulses. This is necessary in order to align the proton magnetization of interest with the spin-lock axis. Unaligned magnetization, as the one of isolated protons, is destroyed by the B_1 field inhomogeneities. The carbon selectivity is achieved through the DANTE-Z[12] method. The signal originating from a scan in which the tip angle β of the soft carbon pulse is 180° is substracted to the one obtained with $\beta = 0°$. Only the signal due to the proton attached to the on-resonance carbon nucleus will thus be present in the final spectrum. The

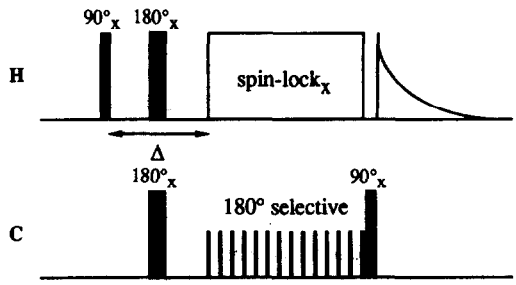
switch between the two β values and the signals' subtraction are performed by the phase program.

Figure 1.



The soft DANTE pulse train is made of an even number of identical hard pulses separated by equal delays, the phases of the individual pulses being either equal for a maximum effect (β = 180°), or alternatively opposite to yield an overall null effect (β = 0°). The calibration of the selective inversion pulse is performed by the pulse sequence of Figure 2, and preferencially with a ^{13}C enriched sample (as 1-^{13}C-(D)-glucose in D_2O or its penta-acetate in organic solvents). Increasing the individual DANTE pulses length, starting from 0, leads to an antiphase doublet of first increasing and then decreasing amplitude (as the variation of the sine function). The inversion condition is found at the first signal cancellation.

Figure 2.



The sequence has been tested on a 200 mM sample of strychnine in $CDCl_3$. The Figure 3 illustrates the scope and limitations of the method. A part of the proton spectrum is given for reference (trace a). The corresponding part of the ^{13}C spectrum is drawn of Figure 4. Really close carbon resonances, as those of C-2 and C-3 ($\Delta\delta$ = 0.15 ppm, $\Delta\nu$ = 11.8 Hz) are necessarily difficult to individualize, resulting in simultaneous excitation of H-2 and H-3 signals (trace 3c). The situation is less critical for C-6 and C-22 ($\Delta\delta$ = 0.34 ppm, $\Delta\nu$ = 25.6 Hz). Slight differences in intensity between traces 3g and 3h allow to discriminate between the protons bound to each of these carbon atoms. The inversion sequence used for these experiments lasts 10ms, and longer durations would result in an increase of selectivity. However C-5 and C-16 ($\Delta\delta$ = 2.32 ppm, $\Delta\nu$ = 175 Hz) are here perfectly well separated. Such subspectra may be produced by extracting F2 slices in a regular HMQC spectrum, but the resolution will be not as good as the one resulting from the 1D experiment. The proton-proton spin coupling patterns are here clearly suitable for analysis. Notice that the superimposed proton signals around 3.2 ppm are clearly resolved.

Traces 3b-j required 1024 scans each. The preparation period is a 1.5 s relaxation delay. The DANTE pulse train contains 90 pulses (2.1 μs each) separated by 120 μs delays. The one-bond ^{13}C-^{1}H coupling constants is assumed to be 150 Hz, the Δ delays is thus set to 3.33 ms (1/2J). GARP[13] decoupling is used during acquisition (8K points). A 0.3 Hz line broadening filter is applied in order to reduce the noise level. A more complete elimination of isolated spins signals is possible using a BIRD-relaxation preparation period, but that was not necessary in the present example. This would be especially useful if the proton spectrum contained intense and sharp resonance lines, for which cancellation may be imperfect due to hardware instability.

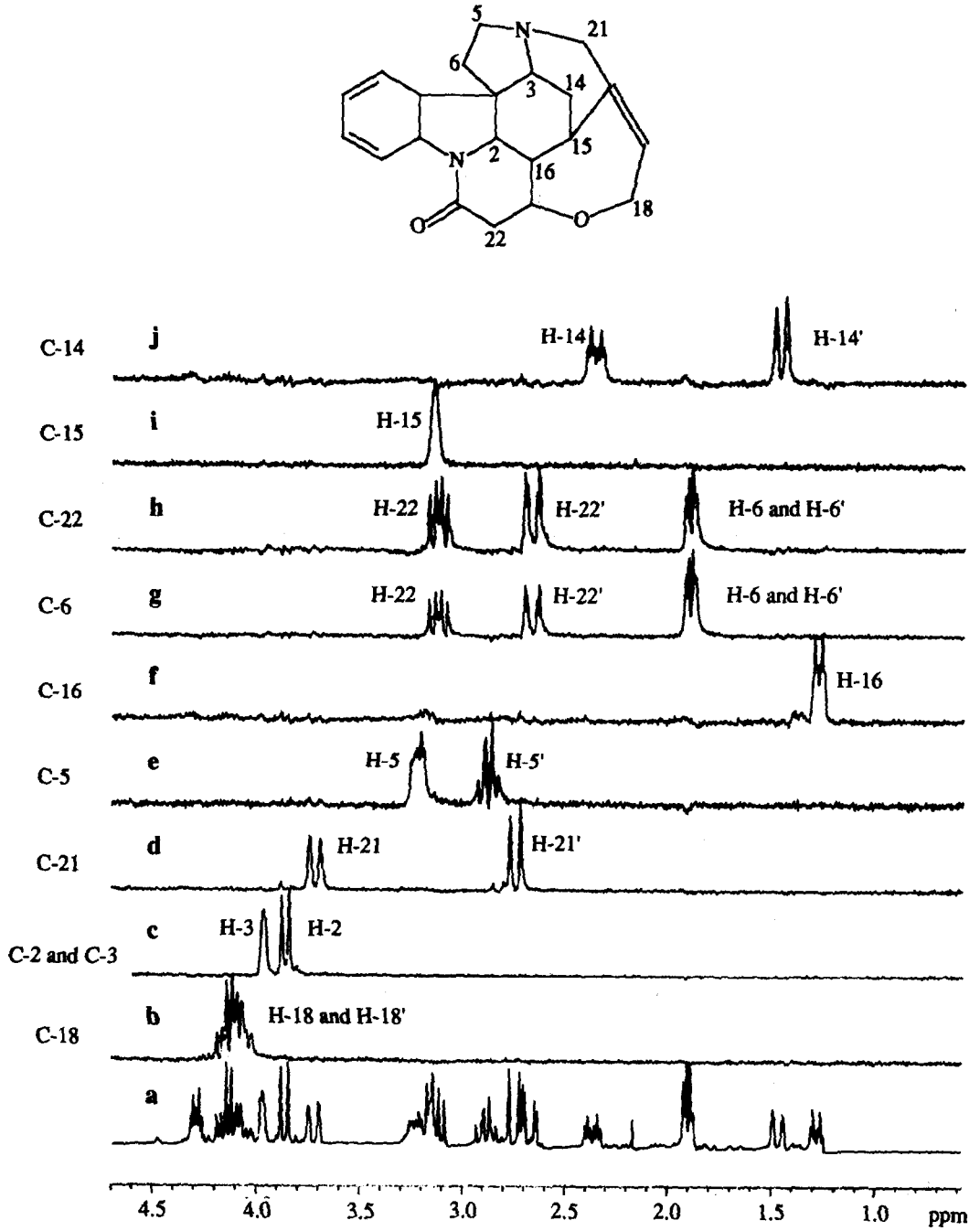


Figure 3. Trace a : high-field region of the proton spectrum of strychnine (structure above).
Traces b-f : Signals of the proton(s) attached to the selected carbon atoms.

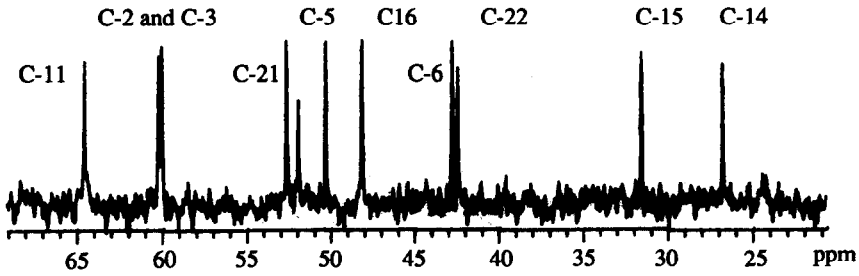


Figure 4. Carbon-13 spectrum of strychnine (aliphatic part)

The experiments were carried out on a Bruker AC300 spectrometer equiped for inverse detection, including the accessory for heteronuclear decoupling, which is also used for DANTE pulse delivery. There is no need of a waveform generator, although similar results could be obtained by replacing the 180° DANTE by a shaped 180° pulse. The pulse sequences described here are available upon request to the authors.

The building block made of the simultaneous soft inversion and spin-locking pulses has already been used in more structurally informative sequences, involving proton magnetisation transfer by isotropic mixing, homonuclear polarisation transfer or nuclear Overhauser effect. These applications, as well as the measurement of long-range heteronuclear coupling constants and relaxation times, will be published in due course.

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