A SELECTIVE INEPT EXPERIMENT FOR THE ASSIGNMENT OF NMR LINES OF LOW-GYROMAGNETIC RATIO NUCLEI THROUGH LONG-RANGE COUPLINGS

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A selective variant of the standard INEPT experiment is suggested. The selectivity is achieved by replacing the refocusing proton pulses of the standard INEPT pulse sequence with selective (DANTE) 180° pulses. Since this approach eliminates the undesirable influences of homo- and heteronuclear couplings, the sensitivity of the method is high. In the case of assigning ^{29}Si NMR lines of trimethylsilylated compounds the pulse sequence can be further simplified and a pair of refocusing pulses can be eliminated from the refocusing period. Advantages of the simplified method are demonstrated.

When protons or other high-gyromagnetic ratio (γ) and high-abundance nuclei are spin–spin coupled to low-γ nuclei the NMR spectra of the latter nuclei are efficiently measured directly by techniques employing a general polarization transfer (INEPT, ref.[1] and DEPT. ref.[2]). In such cases it is often possible to assign the lines in the spectrum by the use of a heteronuclear two-dimensional chemical shift correlation experiment[3]. However, low sensitivity of these experiments makes them very demanding both on the spectrometer time and amount of the sample.

Since typical molecules contain usually only a few nonequivalent low-γ nuclei and since the lines of protons coupled to these nuclei have usually characteristic chemical shifts, the lines can be assigned more efficiently by some suitable selective polarization transfer experiment. This would not only save the spectrometer time but it would also enable measurements of diluted samples.

Selective polarization transfer (or inversion) (SPT, ref.[4] or SPI, ref.[5]) experiments require detailed knowledge of the spectra (e.g. knowledge of the frequencies of the satellite lines in the proton spectrum) and so they are not very suitable for practical applications. Much more promising in this respect are modifications of the general INEPT or DEPT pulse sequencies which can be constructed in such a way that they are selective and yet retain the generality of the parent experiments to the extent that no detailed knowledge of the spectra would be required prior to the experiment.

In the present communication we describe one type of such a selective INEPT experiment which is particularly suited for the above described purpose.

EXPERIMENTAL

The NMR experiments were performed on a Varian XL-200 spectrometer equipped with a V77--220 data system. The operating frequencies were 200 MHz and 39·7 MHz for the ^{1}H and the ^{29}Si NMR measurements, respectively.

The sample, 3α,12α-bis(trimethylsiloxy)-5β-cholanate, was prepared by trimethylsilylation of the parent diol by bis(trimethylsilyl)acetamide as described elsewhere[6]. The measured deuteriochloroform solution (0·7 ml in an NMR tube with o.d. 5 mm) contained 25 mg of the silylated derivative and 1% (v/v) of hexamethyldisilane (HMDSS) which served as an internal ^{29}Si NMR reference (δ −19·79).

RESULTS AND DISCUSSION

The desired selectivity of INEPT experiments can be achieved in a number of ways, several of them have already been reported[7]. Thus the regular pulse sequence of refocused INEPT (refs[1,8–10]) for the measurement of the spectra of nuclei X (*1*) can be made selective in a very simple way just by replacing all the "hard" nonselective proton pulses by "soft" pulses as proposed by Bax for the measurement of long--range couplings[11] or for detection of nonprotonated ^{15}N nuclei[12].

$$\begin{array}{lllllllll} {}^1\text{H:} & 90° - \Delta/2 - & 180° & - \Delta/2 - & 90° & - \Delta_2/2 - & 180° & - \Delta_2/2 - & \text{decouple} \\ \text{X:} & & 180° & & 90° & & 180° & & \text{acquire} \end{array} \qquad (1)$$

This all-proton-pulses-selective variant eliminates dephasing of the desirable coherences under the influences of homonuclear (during Δ delay) and heteronuclear (during Δ_2 delay) couplings. The same effect can be, however, achieved if all 90° proton pulses are retained as "hard" pulses and only the refocusing 180° proton pulses are made selective.

When the coupling constants are small, which is often the case (e.g. assignment of lines of low-γ nuclei through long-range couplings), the selective pulses can be performed as a short (DANTE) series of hard pulses[13]. The proposed selective pulse sequence then can be written as

$$\begin{array}{lllll} {}^1\text{H:} & 90° - \Delta/2 - & (180°/n - \tau -)_n & - \Delta/2 - & 90° - \\ \text{X:} & & 180° & & 90° \end{array}$$

$$\begin{array}{llll} \Delta_2/2 - & (180°/n - \tau -)_n & - \Delta_2/2 - & \text{decouple} \\ & 180° & & \text{acquire} \end{array} \qquad (2)$$

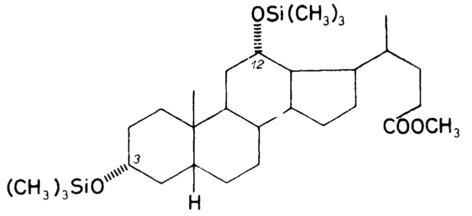
where the number of pulses in the DANTE series n and the delay between them τ should be chosen so that the X-satellites in the ^{1}H NMR spectrum of the chosen proton are affected by the 180° proton pulse. The outcome of the pulse sequence (*2*) depends strongly on the quality of the last proton refocusing pulse.

In order to avoid this negative influence it is often advantageous to eliminate the last pair of the refocusing pulses from the sequence. Then, the simplified sequence is:

$$\begin{array}{llllll} {}^1\mathrm{H}: & 90^\circ - \Delta/2 - & (180^\circ/n - \tau -)_n & - \Delta/2 - & 90^\circ - \Delta_2 - & \text{decouple} \\ \mathrm{X}: & & 180^\circ & & 90^\circ & \text{acquire} \end{array} \qquad (3)$$

The sensitivity of this modification is reduced by additional couplings between nuclei X and any other proton not involved in the polarization transfer. In such a case the delay Δ_2 should be optimized. Of course, with this simplified sequence it is necesary to adjust the phase of each resulting spectrum individually.

Application of sequence (*3*) to the assignment of two lines in the ^{29}Si NMR spectrum of a trimethylsilylated diol is presented in Fig. 1. In this example the mutliplets in ^{1}H NMR spectrum (bottom trace) which are due to H-12 and H-3 protons were identified by their distinct chemical shifts and they were differentiated



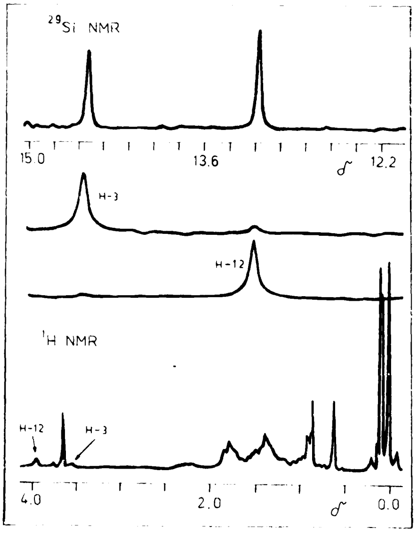


FIG. 1

NMR Spectra of 3α,12α-bis(trimethylsiloxy)-5β-cholenate. Top trace — ^{29}Si NMR spectrum recorded using the routine INEPT pulse sequence. Two middle traces — ^{29}Si NMR spectra recorded using the selective INEPT pulse sequence (*3*) with the DANTE train centered on the multiplets of protons indicated. Bottom trace — ^{1}H NMR spectrum with multiplets due to H-3 and H-12 protons indicated. (Parameters employed in the sequence (*3*): $\Delta = 0{\cdot}140$ s, $\Delta_2 = 0{\cdot}149$ s, $\tau = 0{\cdot}001$ s, $n = 16$, total experimental time of one selective experiment 7 h) by considering the couplings with vicinal protons. In order to assign the (regular) ^{29}Si INEPT spectrum (top trace) two selective INEPT experiments were performed using the pulse sequence (*3*). In these experiments the DANTE trains were centred on H-3 and H-12 proton multiplets. The results (middle traces) are conclusive, the ^{29}Si NMR line at δ 14·6 is due to the silicon on C-3 and that at δ 13·2 is due to the silicon on C-12.

The results clearly demonstrate the advantages of one-dimensional selective INEPT experiment over two-dimensional heteronuclear correlations for the assignment of simple spectra of heteronuclei.

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