

Introduction

The determination of homonuclear and heteronuclear coupling constants is of prime importance in the structural and conformational studies of molecules in solution. Not only do J-couplings contain information about chemical connectivity, they also contain structural information as known for the typical Karplus-like dependence that exhibits 3J vs dihedral angles.

We propose a modified P.E.HSQC experiment¹, referred here as P.E.HSQMBC, for the simultaneous measurement of multiple $J(HH)$, $^1J(CH)$ and $^nJ(CH)$ coupling constants. In this long-range 1H - ^{13}C correlation experiment, the large heteronuclear $^1J(CH)$ coupling constant is employed for separating two multiplet patterns along the indirect F1 dimension while homonuclear HH couplings can be accurately measured from their relative E.COSY displacements in the detected F2 dimension. Furthermore, it will be shown that both E.COSY and IPAP principles can be implemented into the same pulse scheme to simultaneously measure different coupling constants from the same 2D multiplet with an extreme simplicity.

Methodology

Fig. 1 shows the pulse sequences used in this work: A) non-refocused fully-coupled P.E.HSQMBC sequence; B) the same scheme but incorporating an $^1J(CH)$ -scalable BIRD element that allows the selective evolution of $^1J(CH)$ whereas refocuses all $^nJ(CH)$ components during the t_1 period; C) Refocused IPAP version based on the separate acquisition of In-Phase (IP: $\epsilon=on$) and Anti-Phase (AP: $\epsilon=off$) data that are further combined (IP \pm k*AP) to provide separate α - and β -P.E.HSQMBC spectra. A selective 180° pulse is applied in the INEPT period everywhere to remove any $J(HH)$ modulation.

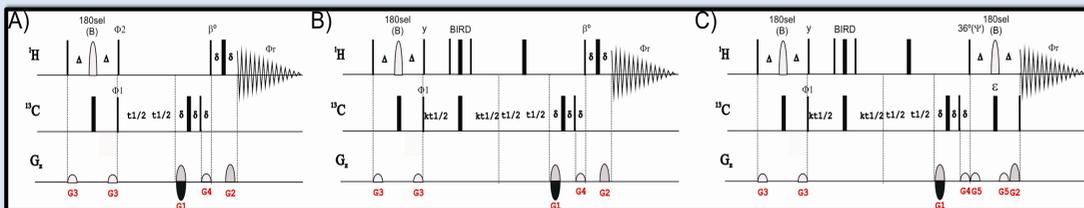


Figure 1

The multiplet patterns obtained from sequences of Fig. 1A, 1B and 1C, respectively are displayed in Fig. 2. Filled and open circles represent multiplet components with opposite phase. In all cases, $J(HH)$ is measured from the E.COSY pattern. On the other hand, $^nJ(CH)$ must be extracted from the analysis of anti-phase multiplet patterns in the same row (in A and B), or from the relative displacement between complementary α/β cross-peaks in two separate spin-state selected spectra (see C).

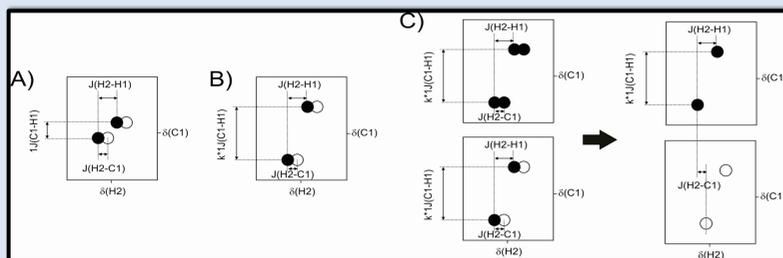
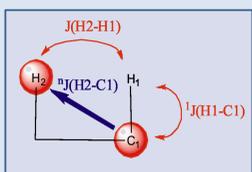


Figure 2

Experimental

As an example, Fig. 3 shows the general protocol followed to obtain separate P.E.HSQC and P.E.HSQMBC spectra after selective refocusing of H15b proton (resonating at 2.35ppm) of strychnine using the scheme of Fig. 1B. Two different data are independently acquired only changing the phase of the 90° proton pulse just applied after the t_1 period (A) using $\phi_2=\gamma$ and (B) $\phi_2=-\gamma$ whereas other relevant phase remain unchanged ($\phi_1=x, -x$ and $\phi_r=x, -x$). After addition and subtraction of these data, two separate C) P.E.HSQC and D) P.E.HSQMBC spectra arising are obtained that allow the determination of all relevant $^1J(CH)$, $J(HH)$ and $^nJ(CH)$ values involving the H15b proton. The factor scaling was set to $k=3$ and the experiment was optimized to 8 Hz.

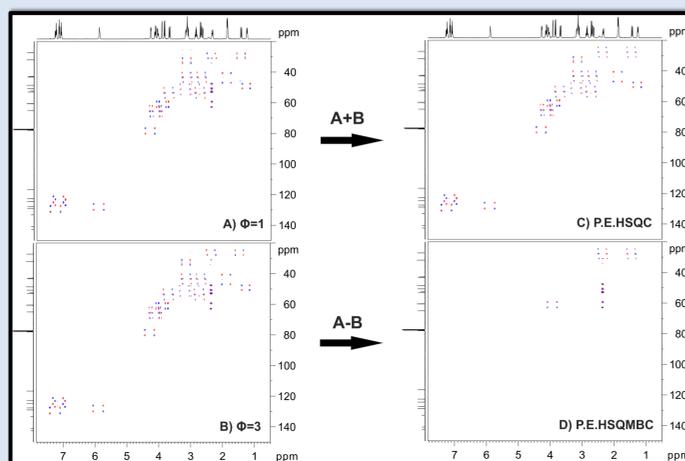
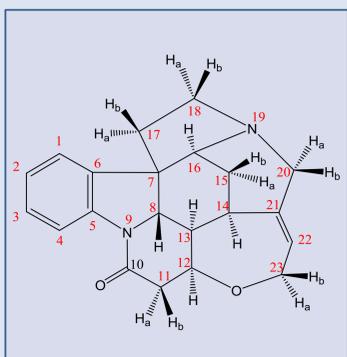


Figure 3

Fig. 4 shows the expanded region of the column corresponding to the H15b proton (see Fig. 3D). Note that from the direct correlation, the relative sign between $^2J(HH)$ and $^1J(CH)$ can be obtained. From the analysis of other cross-peaks, the sign and magnitude of $^3J(HH)$ can be extracted from the E.COSY pattern whereas the analysis of a specific row can provide $^nJ(CH)$ after the application of a conventional fitting procedure from a suitable peak reference.

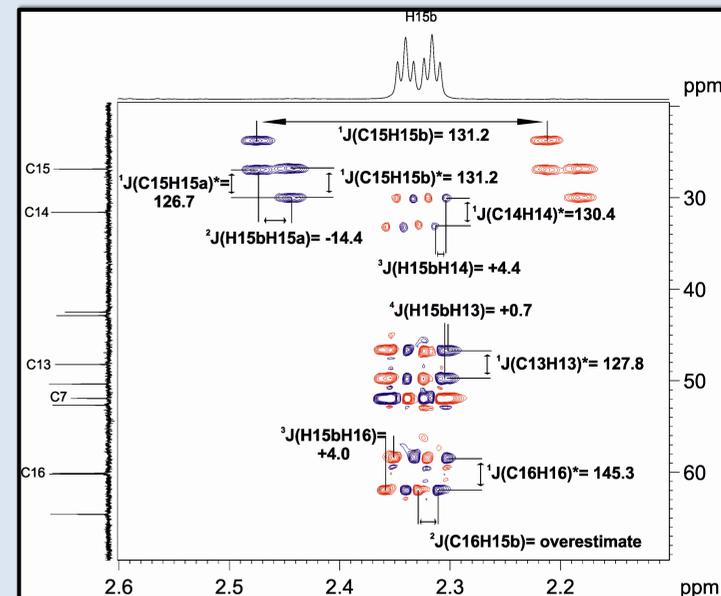


Figure 4

Fig. 5 shows how works the IPAP methodology: complementary IP (Fig. 5A) and AP (Fig. 5B) data are separately acquired and their sum and difference (Fig. 5C and 5D) generate the upfield and downfield components of the active doublet making it possible the straightforward measurement of $^nJ(CH)$ by analyzing the relative displacement between them. In analogy to the regular selHSQMBC experiment the sign information of $^nJ(CH)$ is not available from this analysis.

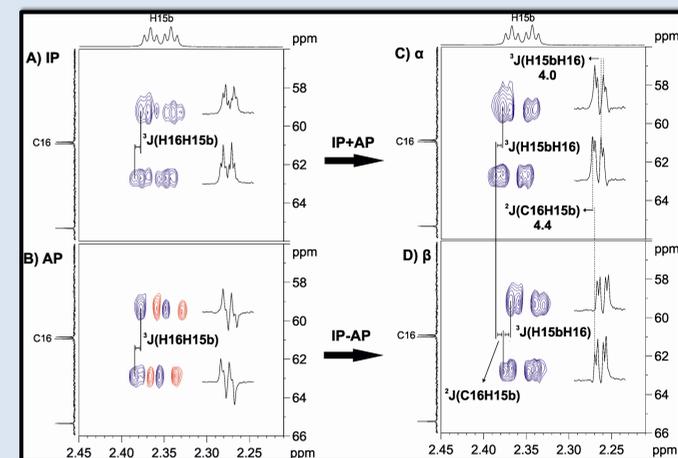


Figure 5

The suggested methods can also be successfully applied to organophosphorus (or organofluorine) compounds in order to simultaneously measure the sign and the magnitude of $J(HP)$ and $J(CP)$ (besides $J(HH)$) from the additional E.COSY pattern generated from the passive heteronucleus (Fig. 6).

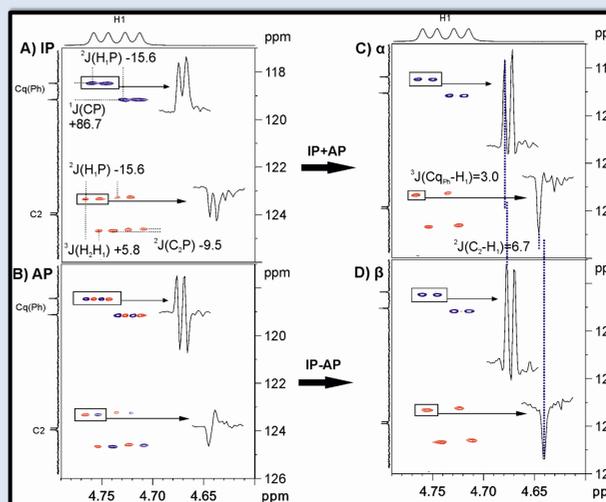
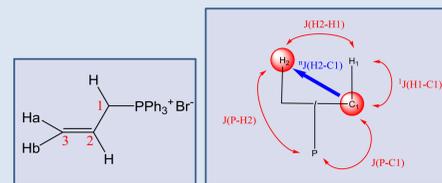


Figure 6



Conclusions

- It has been that the concepts of J-resolved, E.COSY and IPAP principles can be mixed all together into the same pulse scheme in order to measure multiple coupling constants from a single 2D cross-peak analysis.
- The sign and the magnitude of proton-proton coupling constants are measured along the direct dimension from the relative E.COSY multiplet pattern displacement due to the passive one-bond coupling constant splitting generated in the indirect dimension.
- Long-range proton-carbon coupling constants are independently determined in the detected dimension from a traditional fitting analysis of antiphase multiplet patterns or, more conveniently, from the IPAP multiplet displacement observed in a refocused experiment.
- The sign and the magnitude of additional heteronuclear couplings can simultaneously be measured in organophosphorus or organofluorine derivatives