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A Simple Method for Measuring Long-Range $^{1}H-^{13}C$ Coupling Constants in Organic Molecules

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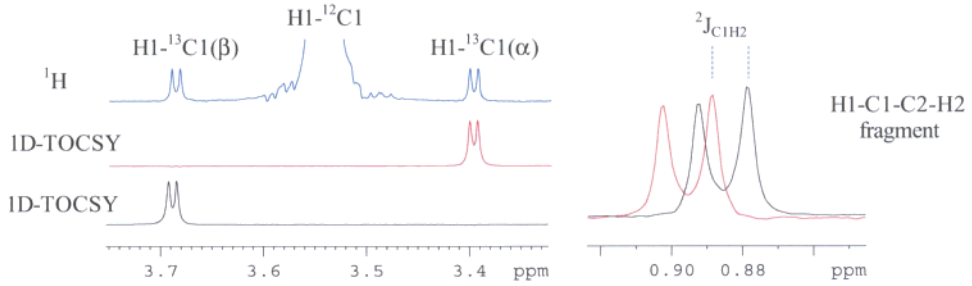

This study presents a simple method for measuring long-range heteronuclear coupling constants between protons and proton-bearing carbons. The approach involves recording two conventional 1D-TOCSY experiments in which the offset of the selective proton pulse is set on the low- and high-frequency ^{13}C satellites of an isolated proton signal, H*i*. Long-range heteronuclear coupling values between the ^{13}C*i* bonded to H*i* and the protons H*j*,*k*...*n* that belong to the same spin system were easily determined from the relative displacement of the relayed H*j*,*k*...*n* signals in the satellite-selective TOCSY spectra. The sense of the displacement indicated the sign of the coupling constants.

Introduction

Heteronuclear $^{1}H-^{13}C$ coupling constants over more than one bond ($^{n}J_{C,H}$; $n > 1$) are extremely useful for solving stereochemical problems.[1] A method, denoted as "*J*-based configuration analysis", has been developed to elucidate the stereochemical configuration of acyclic organic compounds based on carbon−proton spin-coupling constants ($^{2,3}J_{C,H}$).[2] The use of long-range $^{1}H-^{13}C$ coupling constants to determine the structure of organic molecules is still uncommon, however, and the combination of homonuclear $^{3}J_{H,H}$ and NOEs is usually used instead.[3] In addition to problems associated with the low sensitivity of ^{13}C nuclei, this is probably due to the absence of a standard and convenient method for correlating long-range H−C couplings and structure. As a consequence, the application of this NMR parameter to the configurational and conformational assignment of small organic compounds is not frequently utilized.

A number of two-dimensional (2D) NMR experiments designed for measuring long-range $^{n}J_{C,H}$ values have been reviewed.[4] Two-dimensional experiments have long acquisition times imposed by their requirement for high digital resolution in F_1, however, and are not suited for the task of measuring specific couplings to a given carbon. Since the solution to a particular stereochemistry problem for small to medium molecules often lies in a limited number of heteronuclear coupling constants, 1D NMR experiments acquired in a shorter time and providing higher resolution are therefore more attractive. Most 1D pulse sequences for measuring long-range heteronuclear couplings are inverse experiments that usually include at least one selective carbon pulse.[5] Unfortunately, these nonstandard pulse sequences are often only used by specialists and seldom by practicing organic chemists. Thus, a simple approach for

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(1) Eberstadt, M.; Gemmecker, G.; Mierke, D. F.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1671−1695.
(2) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876.
(3) (a) Eberstadt, M.; Mierke, D. F.; Kock, M.; Kessler, H. *Helv. Chim. Acta* **1992**, *75*, 2583−2592. (b) Koeck, M.; Junker, J. *J. Org. Chem.* **1997**, *62*, 8614−8615.
(4) Marquez, B. L.; Gerwick, W. H.; Williamson, R. T. *Magn. Reson. Chem.* **2001**, *39*, 499−530.

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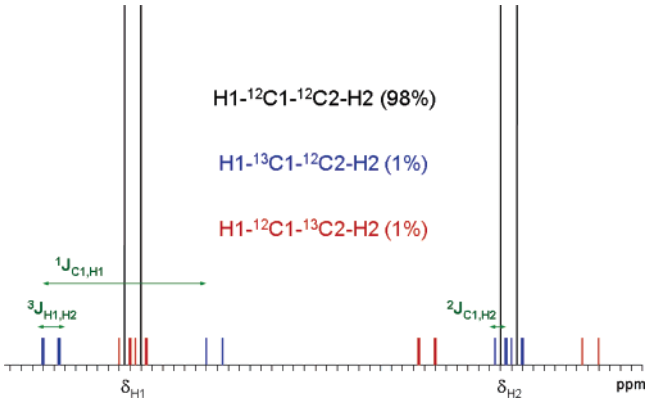


FIGURE 1. Schematic representation of the ^{1}H spectrum in the model system, H1−C1−C2−H2. The signals are color coded according to the isotopomer to which they correspond. The thick and thin lines of the minor isotopomers represent ^{13}C in its β or α state, respectively. For simplicity, the intensity of each signal is not scaled and the small $^{13}C/^{12}C$ isotopic effect on the ^{1}H chemical shifts that prevents the satellites from being symmetrically placed around the main peak is neglected. The isotopomer with two ^{13}C atoms is not considered because of its low abundance (approximately 0.01%).

measuring $^{n}J_{C,H}$ values would be highly beneficial to extend the use of these couplings to the structural elucidation of organic compounds.

In this study, we develop a satellite-selective 1D-TOCSY experiment that would measure the sign and the magnitude of $^{n}J_{C,H}$ in small organic molecules in a fast, sensitive, and accurate way. Lunazzi and Mazzanti,[6] who demonstrated the use of a satellite-selective 1D-NOESY experiment to elucidate symmetric isomers, inspired our approach. The selective inversion of the ^{13}C satellites in a 1D-NOESY experiment[7] yielded NOE effects on the spatially close ^{12}C-bonded proton of the same isotopomer, resulting in the unambiguous structural assignment of configuration in symmetric molecules. Rather than simultaneous irradiation of both satellites, we examined the individual selective inversion of each satellite resonance followed by a spin-lock period in two individual 1D-TOCSY experiments. The 1D-TOCSY is a standard NMR pulse sequence that yields 1D subspectra of protons that are part of the same spin system.[8]

Let us consider the effect of this approach on a model spin system, H1−C1−C2−H2, in which H1 is coupled to H2 and $^{3}J_{H1,H2} > 0$. For simplicity, the $^{12}C/^{13}C$ isotopic effect on the chemical shifts is neglected and a first-order analysis is assumed. Ten doublets are expected in the ^{1}H spectrum: two doublets at δ_{H1} and δ_{H2} for the most abundant isotopomer, H1−^{12}C1−^{12}C2−H2 (ca. 98% natural abundance), four doublets for the minor isotopomer, H1−^{13}C1−^{12}C2−H2 (ca. 1% natural abundance), two at $\delta_{H1} \pm {}^{1}J_{C1,H1}/2$ that are ascribed to H1 and two at $\delta_{H2} \pm {}^{2}J_{C1,H2}/2$ that are ascribed to H2, and four doublets for the minor isotopomer, H1−^{12}C1−^{13}C2−H2 (ca. 1% natural abundance) (Figure 1). Minor signals that are separated by one-bond H−C coupling, commonly denoted as the ^{13}C satellites, are often observed in the ^{1}H spectrum. Other minor signals separated by the two-bond H,C couplings cannot be detected because they are obscured by the strong signal of the ^{12}C-bonded protons in the predominant isotopomer. The selective excitation of each H1 satellite followed by magnetization transfer to the coupled H2 proton in the 1D-TOCSY experiments would give

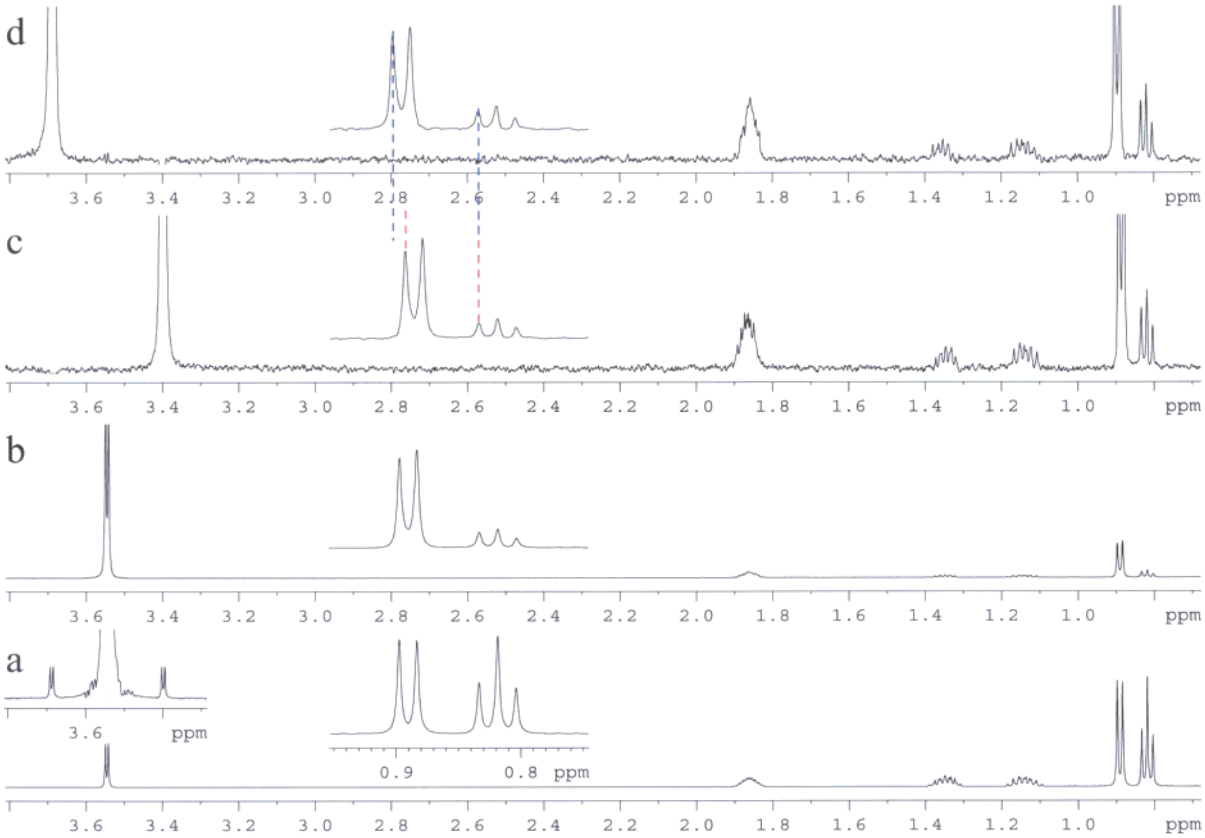


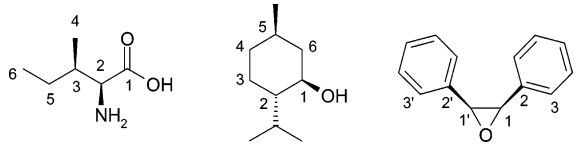
FIGURE 2. 500 MHz spectra of isoleucine in D_2O. (a) Conventional ^{1}H spectrum; (b−d) 1D-TOCSY spectra with selective inversion of the ^{12}C-bonded H2 resonance (b), the low-frequency H2 satellite (c), or the high-frequency H2 satellite (d). Displacement of the signals is illustrated by expansion of the methyl region. The acquisition time of each satellite-selective 1D-TOCSY was 17 min.

rise to signals at $\delta_{H2} \pm {}^2J_{C1,H2}/2$ in the absence of a major signal at δ_{H2}, allowing ${}^2J_{C,H}$ to be measured by comparing the two highly resolved 1D spectra.

Results and Discussion

As a proof of concept, we tested the methodology on a natural amino acid isoleucine (**1**). The heteronuclear coupling constants between the α-carbon and γ-protons of amino acids and the homonuclear coupling constants between the β- and γ-protons help to characterize the side-chain dihedral angle χ^2 in peptides.[9]

The ^{1}H spectrum of isoleucine showed the ^{12}C-bonded H2



1 **2** **3**

signal at 3.54 ppm and its ^{13}C-bonded satellite peaks at ca. ±72 Hz (Figure 2a). The conventional 1D-TOCSY spectrum in which the ^{12}C-bonded H2 resonance was selectively inverted and its magnetization transferred to the remaining protons on the isoleucine side chain is shown in Figure 2b. When the low- or the high-frequency satellite was used as the starting point for magnetization transfer, the resulting TOCSY spectra exhibited signals which arose from the proton bonded to ^{13}C2 in its α or β spin state, respectively (Figure 2c and d). These signals were displaced with respect to their position in the conventional 1D-TOCSY experiment. Displacement between the TOCSY peaks was clearly observed for the doublet that corresponded to γ-methyl H4 protons that are three bonds away from C2. In contrast, no displacement was detected for the triplet corresponding to the δ-methyl H6 protons that are four bonds away from C2 because the H,C coupling constants between nuclei that are separated by more than three bonds in noncyclic aliphatic molecules are close to zero.[10] The values of the ${}^nJ_{C2,Hi}$ couplings were then measured from the relative displacement between the signals of the two satellite-selective 1D-TOCSY experiments (Table 1). The sign of the coupling was deduced from the sense of the displacement. Thus, if the displacement exhibited the same sense as that of the large one-bond coupling, the coupling constants were positive, while if the displacement exhibited the opposite sense, the constants were negative.

To provide a second example, we measured the long-range heteronuclear coupling constants between the C1 of menthol (**2**) and the rest of its protons using the satellite peaks of H1 as the source of magnetization transfer. In this case, the spectra were acquired using a high-sensitive cryoprobe. This technology is 4-fold more sensitive than conventional probes and dramatically reduces acquisition times. The spectra recorded for menthol are shown in Figure 3a. H1 appeared at 3.43 ppm, and its satellite peaks, not visible at the intensity level shown in Figure 3a, could be selectively inverted (Figure 3b and c). We found that a 60 ms 180° Gaussian pulse was suitable for inverting the satellites without affecting the central peak. When this block was followed by a spin-lock period, the corresponding β and α 1D-TOCSY subspectra were obtained with only 64 scans (Figure 3d and e) from which the heteronuclear coupling constants could be measured in the manner described for **1** (Figure 4). The values were similar to previous values reported for menthol.[5g]

We also examined *cis*-stilbene oxide **3** to illustrate the application of our methodology to ${}^2J_{C,H}$ measurements in symmetric molecules. The signal of the chemically equivalent H1 and H1′ protons of the predominant isotopomer, with two ^{12}C atoms in the epoxide ring, appeared as a singlet at 4.37 ppm, while the satellite signals arising from the minor isotopomer, with one ^{13}C atom in the epoxide ring, appeared as

TABLE 1. Long-Range H,C Couplings between the α-Carbon and the Side-Chain Protons in 1

^{1}H−^{13}C pair	^{1}H−^{13}C distance (bonds)	long-range coupling (Hz)
H3−C2	two	−4.2
H4−C2	three	+5.0
H5−C2	three	+3.4
H5′−C2	three	+3.1
H6−C2	four	~0.0

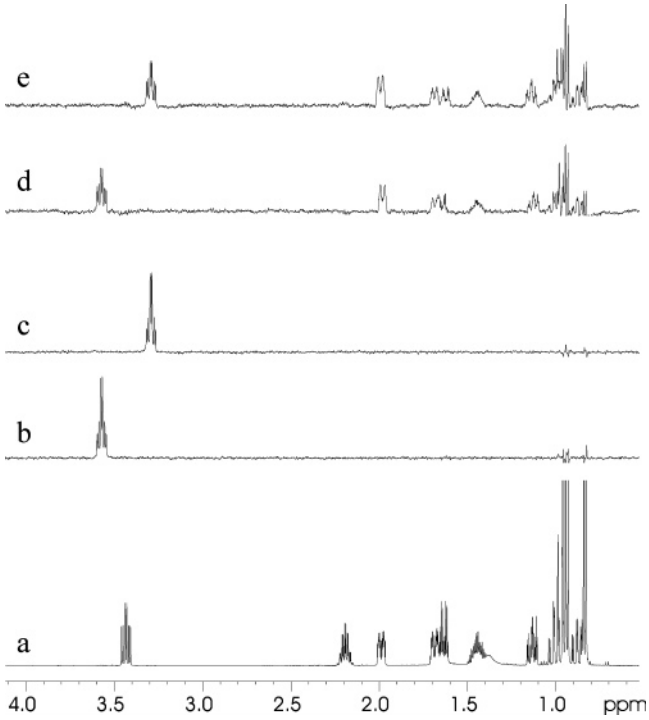


FIGURE 3. 500 MHz spectra of menthol in $CDCl_3$. (a) Conventional ^{1}H spectrum; (b−e) selective excitation of the high-frequency (b,d) or low-frequency (c,e) satellites and subsequent acquisition with (d,e) or without (b,c) a spin-lock period between satellite excitation and acquisition. The acquisition time of each satellite-selective 1D-TOCSY was 3 min.

(5) (a) Nuzillard, J.-M.; Freeman, R. *J. Magn. Reson. A* **1994**, *110*, 262−265. (b) Fukushi, E.; Kawabata, J. *J. Magn. Reson. A* **1994**, *108*, 103−105. (c) Bazzo, R.; Barbato, G.; Cicero, D. O. *J. Magn. Reson. A* **1995**, *117*, 267−271. (d) Yang, D.; Nagayama, K. *J. Magn. Reson. A* **1996**, *118*, 117−121. (e) Fäcke, T.; Berger, S. *J. Magn. Reson. A* **1996**, *119*, 260−263. (f) Parella, T.; Belloc, J. *J. Magn. Reson.* **2001**, *148*, 78−87. (g) Parella, T.; Belloc, J.; Sánchez-Ferrando, F. *Magn. Reson. Chem.* **2004**, *42*, 852−862.

(6) Lunazzi, L.; Mazzanti, A. *J. Am. Chem. Soc.* **2004**, *126*, 12155−12157.

(7) Stott, K.; Stonehouse, J.; Keeler, J.; Hwang, T.-L.; Shaka, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 4199−4200.

(8) (a) Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 7197−7198. (b) Adell, P.; Parella, T.; Sanchez-Ferrando, F.; Virgili, A. *J. Magn. Reson. B* **1995**, *108*, 77−80. (c) Fäcke, T.; Berger, S. *Tetrahedron* **1995**, *51*, 3521−3524.

(9) Sattler, M.; Schwalbe, H.; Griesinger, C. *J. Am. Chem. Soc.* **1992**, *114*, 1126−1127.

(10) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*, 3 ed.; VCH: Weinheim, Germany, 1989; pp 134−147.

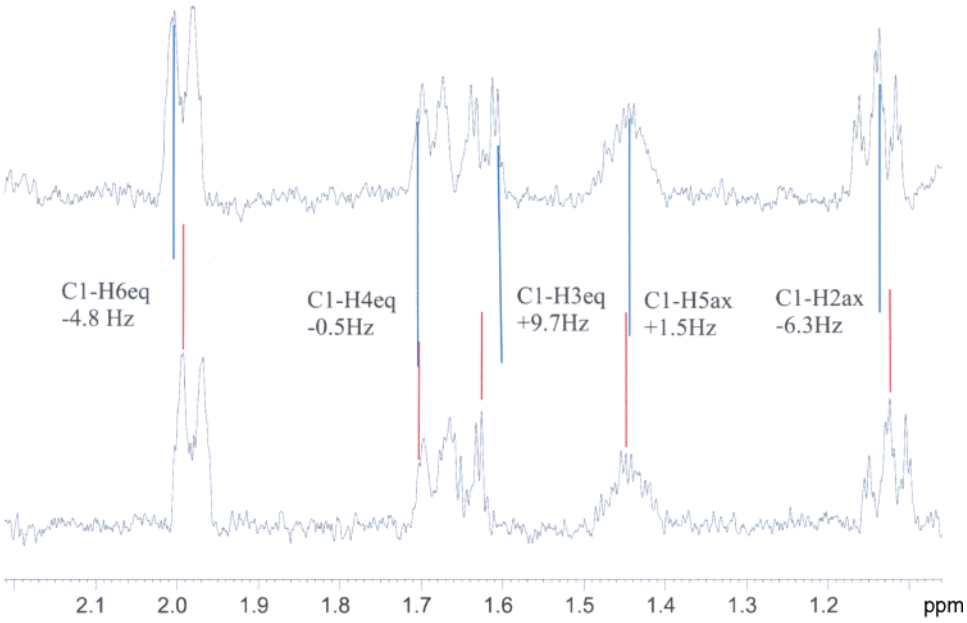


FIGURE 4. Measurement of the long-range C,H couplings between C1 and the H*i*,*j*...*k* protons of **2** by comparing the 1D-TOCSY spectra.

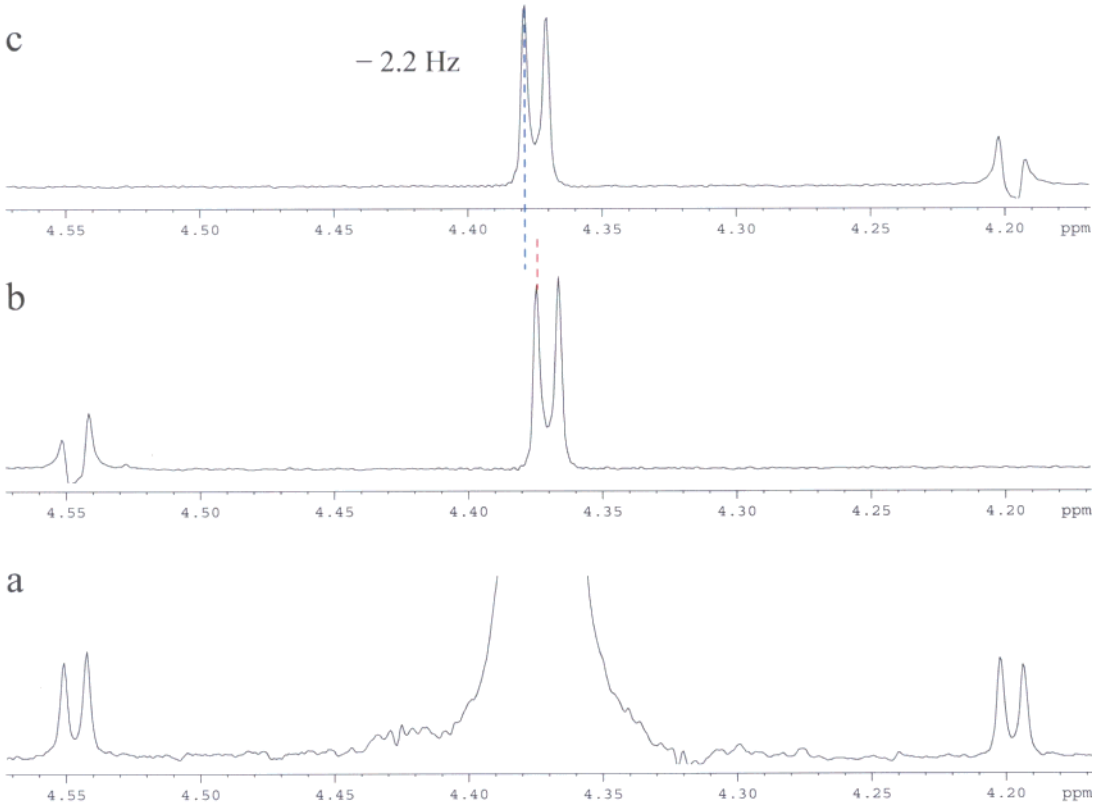


FIGURE 5. 500 MHz spectra of **3** in $CDCl_3$. (a) Expansion of the conventional ^{1}H spectrum; (b,c) satellite-selective 1D-TOCSY spectra. Measurement of the $^{2}J_{C1,H1'}$ coupling is represented. The acquisition time of each satellite-selective experiment was 5 min.

doublets due to the coupling to the vicinal ^{12}C-bonded proton (Figure 5a). The heteronuclear two-bond coupling between C1 and H1′ may be derived from the coupling pattern of the epoxide carbon signal in a coupled ^{13}C spectrum. However, in addition to the longer acquisition times required for the ^{13}C-detected experiments, measurement of the coupling of interest was complicated because the epoxide carbon signal exhibited an extra three-bond H,C coupling with the H3 protons of the phenyl ring. In contrast, comparison of the satellite-selective TOCSY subspectra yielded the coupling of interest in a faster and more accurate manner (Figure 5b and c). The coupling was equal to −2.2 Hz, which was close to its previously reported value (−2.3 Hz).[6]

In addition to its tremendous simplicity, the satellite-selective TOCSY experiment is advantageous over the existing 1D experiments that are based on HSQC or HMQC blocks. Since

this experiment only uses proton pulses and gradients, pulses on the ^{13}C channel are not involved, and so no previous knowledge of ^{13}C frequencies is required. As the $^{1}H-^{13}C$ couplings are measured from signal displacement in 1D-TOCSY spectra, the precision of the values depends on the accuracy with which frequency measurements can be made, which is given by the digital resolution of the 1D spectra. A limitation of the methodology is that it can only be applied to signals that are far enough apart from other resonances in the ^{1}H spectrum in order to achieve selective inversion of the ^{13}C-bonded satellites without affecting ^{12}C-bonded resonances. Since a significant number of proton signals from small organic compounds, such as methine and methylene protons attached to heteronuclei, olefinic protons, and aromatic resonances of heterocycles, may appear in clear regions of the ^{1}H spectrum, it is likely that this methodology can be applied to many cases. Finally, it should also be mentioned that coupling constants to unprotonated heteronuclei are not accessible because of their reliance on TOCSY transfer.

Conclusions

We showed that the comparison of two 1D-TOCSY spectra generated by selective inversion of each ^{13}C satellite of a certain proton signal, H*i*, allows for measurement of long-range heteronuclear couplings between protons of the same spin system as H*i* and the carbon bonded to H*i*. The $^{n}J_{C,H}$ couplings are easily determined from the signal displacement, and values smaller than the line width can be measured with excellent accuracy. The usefulness of this method was shown for an amino acid (isoleucine), a natural product (menthol), and a symmetric compound (*cis*-stilbene oxide).

To the best of our knowledge, this is the simplest scheme that uses proton-detected NMR experiments to determine long-range H,C couplings. Since the standard 1D-TOCSY pulse sequence provided by the vendor can be used in this methodology, it constitutes an attractive option for organic chemists who are not necessarily NMR specialists who wish to use long-range H,C couplings for the configurational assignment of organic molecules. Our methodology would be especially useful for cases in which the homonuclear couplings in tandem with NOE data are not sufficient to solve the problem at hand.

Experimental Section

Isoleucine (**1**), menthol (**2**), and *cis*-stilbene oxide (**3**) were purchased from a commercial source and used without further purification. The compounds (ca. 10 mg) were dissolved in 0.6 mL of D_2O (**1**) and $CDCl_3$ (**2** and **3**) and transferred into an NMR tube. The NMR experiments were acquired on a 500 MHz spectrometer at 25 °C with a 5 mm, inverse, broadband probe head and a *z*-gradient coil (**1** and **3**) or with a 3-channels 5 mm cryoprobe (**2**). The spectra were referenced to the residual solvent signal at 4.79 ppm (D_2O) or 7.26 ppm ($CDCl_3$). The transients of the 1D-TOCSY experiments were 8 when the ^{12}C-bonded signal was inverted and 512, 64, and 128 for **1**, **2**, and **3** respectively, when ^{13}C satellites were inverted. The gradient version of the 1D-TOCSY experiment provided by the vendor was used. The pulse sequence was comprised of an initial hard 90° ^{1}H pulse to excite all the proton resonances followed by the [gradient−selective 180° ^{1}H pulse−gradient] sandwich that retained only the magnetization of the chosen proton, which was then transferred to the protons of the same spin system using a MLEV[11] mixing scheme. The length of the selective ^{1}H pulse (1% truncated Gaussian shape) was 60 ms to achieve the desired selectivity ($\gamma B_1/2\pi = 20$ Hz). A mixing time of 60 ms was used.

Supporting Information Available: A user's guide to the procedure that provides the parameter set used for recording the spectra and a basic step-by-step protocol to perform the experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355−360.