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Application of HMQC in Determination of Chiral Purity Using Quinine

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Abstract: HMQC correlation of diastereomeric salts obtained from complexes with quinine is described. The HMQC/quinine combination is a convenient and potentially general method for chiral purity determination.

The growing interest in chiral drugs[1] occasionally necessitates the rapid and accurate analysis of chiral purity. NMR analysis of diastereomeric salts is an effective and simple method for the determination of optical purity.[2] Recently, there has been interest in using quinine as a chiral solvating agent.[3] Quinine is readily available and inexpensive, and can be used to differentiate enantiomers in a variety of compounds by NMR.[3] One of the drawbacks in using quinine is the complexity of its NMR spectrum, with potential overlap of resonances with the signals from the enantiomers to be evaluated. We have developed a method by which this potential problem can be completely alleviated and the chiral purity of acids can be accurately and conveniently evaluated.

In using a chiral solvating agent for optical purity determination, two conditions must be optimized. First, the peak separation of a signal from the compound to be studied must be sufficient to provide accurate integration and, second, there must be no overlap of the peaks to be analyzed with any peaks from the solvating agent or any other adventitious impurities in solution a problem which is important at less than 500 MHz. This can be problematic and time consuming as the ratio of reagent to substrate must be empirically optimized to obtain suitable data. We have shown that the use of HMQC (heteronuclear, multiple quantum correlation)[4] with a chiral shift reagent eliminates the latter requirement and only the peak separation needs to be considered for chiral analysis.[5]

Shown in Figure 1 is the 1D ^{1}H NMR spectrum for a 1:1 mixture of DL-isocitric lactone (1) and (-)-quinine recorded at 400 MHz. This spectrum exemplifies the potential problem of peak overlap. Analysis clearly shows peak resolution of the methine proton at carbon 1 (Figure 1) sufficient for measurement. Unfortunately, one of the peaks is overlapped with an impurity resonance.

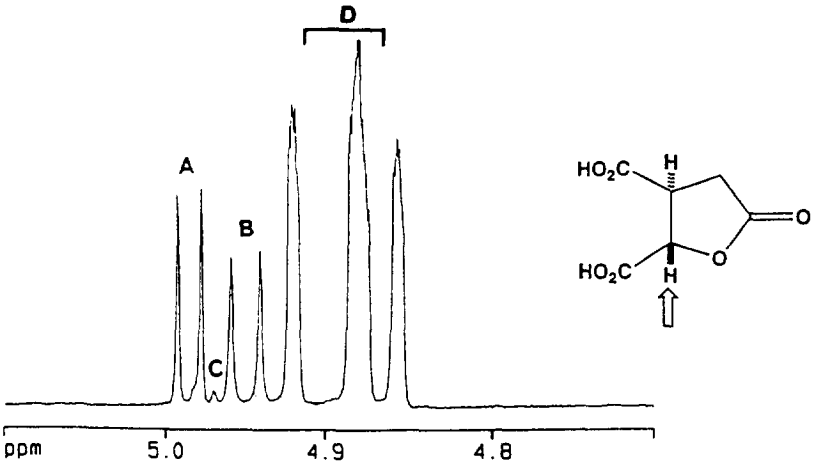

Figure 1. 1D ^{1}H NMR spectrum of DL-isocitric lactone with 1 equivalent of (-)-quinine in $CDCl_3$ at 298 K. Only the methine proton at carbon 1 region is shown. A and B are from DL-isocitric lactone, C arises from an impurity, and the signals at D are quinine resonances.

Carbon-13 chemical shifts generally show greater dispersion than proton chemical shifts and thus allow the proton degeneracies to be resolved using the HMQC experiment. As shown by the HMQC 2D data in Figure 2 for the racemic mixture of DL-isocitric lactone, the overlap of resonances in the proton dimension is completely resolved by the differences in the carbon chemical shifts. The 0.01 ppm and the 0.05 ppm shift differences of the impurity peak and quinine resonances become almost 10 ppm and 40 ppm in the ^{13}C spectrum, providing a dramatic demonstration of the enhanced resolution afforded by this method. Moreover, the doublets A and B are further resolved in the ^{13}C dimension, which might prove useful in situations where resolution in the ^{1}H dimension is suboptimal.

Figure 3 shows the 1D ^{1}H NMR spectrum of an artificially produced 95.0:5.0 mixture of the two enantiomers of 2. Although tert-butyl methyl signals of the isomers split in the presence of (-)-quinine, as shown in the spectrum, it is not obvious which peak should be assigned to the minor enantiomer. It is also difficult to integrate accurately each of the enantiomer peaks since the methyl resonance of the minor enantiomer is too close to the quinine signal. The HMQC spectrum easily resolves the methyl groups from quinine (Figure 4). Integration of the 1D slice of that row yields a 95.2:4.8 ratio of enantiomers. Based on the signal-to-noise ratio (300:1) obtained here, it is estimated that greater than a 99:1 enantiomeric mixture could be readily evaluated.

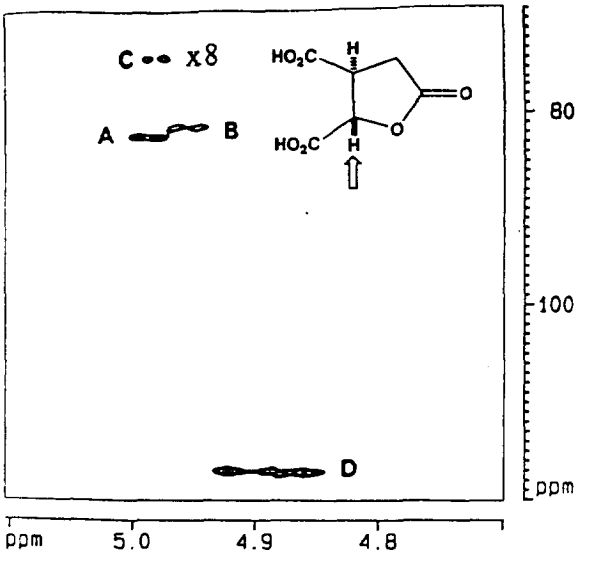

Figure 2. 2D HMQC spectrum of DL-isocitric lactone with 1 equivalent of (-)-quinine in $CDCl_3$ at 298 K. Only the same methine region is shown.

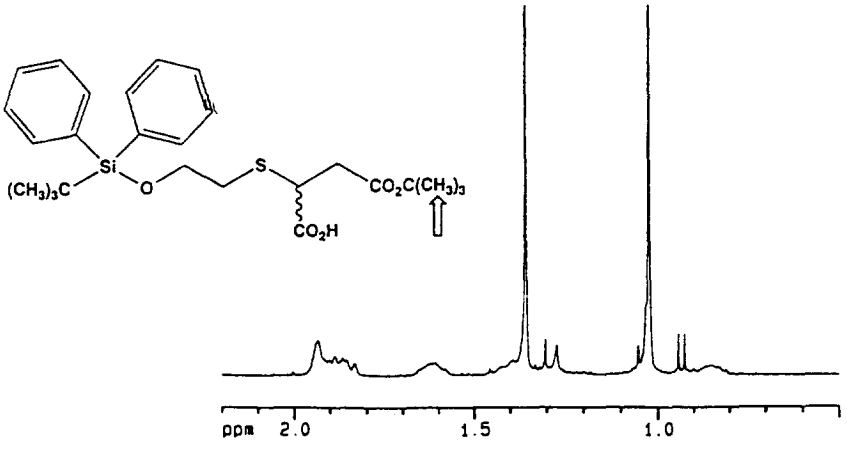

Figure 3. Methyl region of 1D ^1H NMR spectrum of a mixture of enantiomers (95.0:5.0) of 2 with one equivalent of (-)-quinine in $CDCl_3$ at 298 K.

We have demonstrated the utility of HMQC in the evaluation of chiral purity. The method is also successful for determination of chiral purity of racemic samples of ibuprofen, O-acetylmandelic acid, and trans-3-(4-methoxyphenyl)glycidic acid methyl ester (data not shown). The accuracy of the data in all cases was better than 1% as measured from 1D slices through the spectrum.

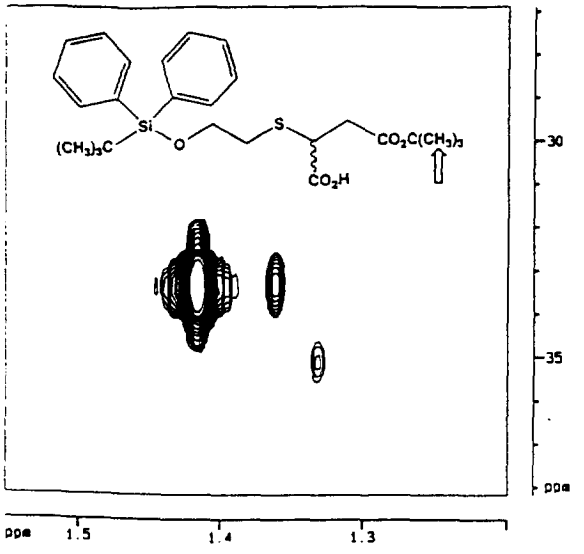

Figure 4. Methyl region of HMQC spectrum of 2 + 1 equiv. (-)-quinine.

The use of HMQC greatly enhances the resolution of the proton NMR spectrum, making integration of peaks straightforward which will be important for chemists not having access to high field strengths. The method is easy to use and yields data that are easy to interpret, sensitive, and accurate.[6]

References and Notes

1. Stinson, S.C. *Chem. Engin. News* **1995**, *73* (41), 44.

2. Parkewr, D. *Chem. Rev.* **1991**, *91*, 1441.

3. (a) Barretta, G.; Mastantuono, A.; Salvadori, P. *Tetrahedron Asymm.* **1995**, *6*, 1965. (b) Rosini, C.; Uccello-Barretta, G.; Pini, D.; Abete, C.; Salvadori, P. *J. Org. Chem.* **1988**, *53*, 4579. (c) Salvadori, P.; Pini, D.; Rosini, C.; Bertucci, C.; Uccello-Barretta, G. *Chirality* **1992**, *4*, 43.

4. Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565.

5. A typical sample was dissolved in $CDCl_3$ and one equivalent of (-) quinine was added. If sufficient separation of any resonances in the 1D spectrum was observed an HMQC spectrum was obtained. HMQC data were obtained using a Bruker 400 MHz DMX spectrometer with mixing time of 350 msec. 4K data points were collected in F_2 and 256 t_1 increments were collected in F_1. The data was collected for 32 scans in each t_1 increment and was apodized using an unshifted sine function in each dimension to produce the best line shape. The signal-to-noise estimate was obtained from a slice through the appropriate row and peak integrations were measured in the usual manner from 1D slice spectra.

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