Micro Inverse-Detection: A Powerful Technique for Natural Product Structure Elucidation

Ronald C. Crouch, and Gary E. Martin

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MICRO INVERSE-DETECTION: A POWERFUL TECHNIQUE FOR NATURAL PRODUCT STRUCTURE ELUCIDATION

RONALD C. CROUCH and GARY E. MARTIN*

Division of Organic Chemistry, Burroughs Wellcome Co., Research Triangle Park, NC 27709

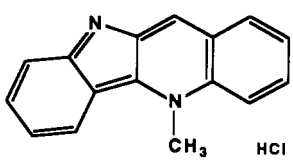
ABSTRACT.—Sample quantities required for heteronuclear correlation spectra are significantly reduced when the experiments are performed in a micro-inverse detection probe. Spectra are reported for a 12 μg sample of cryptolepine HCl [**1**] dissolved in 145 μl of DMSO-d_6. HMQC data shown were recorded in 16 h; acceptable quality data can be acquired in 8 h at this level. Excellent quality HMBC data were acquired on a 35 μg sample in 21 h.

Natural product structure elucidation has seen a number of revolutionary developments in the past fifteen years. The first came with the development and dissemination of 2D nmr techniques that included COSY and ^{1}H-^{13}C HETCOR (1–3). These experiments were complimented by long-range heteronuclear correlation experiments (4,5). In concert, the techniques cited made it possible to elucidate the structures of moderate size (MW 200–600 da) natural products on samples of 20 mg or less.

A second major advance in the elucidation of molecular structure coincides with the development of the inverse- or proton-detected HMQC experiment reported by Bax and Subramanian in 1986 (6) based on earlier work by Müller (7) and by Bax, Griffey, and Hawkins (8,9), followed shortly thereafter by reports of the HMBC (10) and HMQC-TOCSY (11) experiments. Inverse-detected heteronuclear experiments, when performed on a 500 MHz instrument with a contemporary 5 mm inverse probe make it feasible to do complete structure determinations of even complex natural products on a few milligrams. When necessary, HMQC spectra can be readily acquired on submilligram quantities and, with some diligence and attention to experimental detail, on samples as small as 100–150 μg.

Recently, we installed a Z·SPEC® model MID500 micro inverse-detection probe built by Nalorac Cryogenics Corp. which seems to offer the potential to further revolutionize natural product structure elucidation by reducing still further the sample required for a variety of heteronuclear shift correlation experiments. Optimal sample volume in the new probe ranges from 140–160 μl in comparison to the 500–700 μl typically used in a 5 mm probe. In our evaluation of the micro inverse probe's performance, we utilized samples of decreasing quantities of cryptolepine HCl [**1**] dissolved in 145–160 μl of 99.96% DMSO-d_6 (Merck).

The first study affords a comparison of the performance of the micro inverse probe relative to a conventional 5 mm Z·SPEC® model TID500 triple resonance probe. A sample of 200 μg of **1** dissolved in 200 μl of solvent was used to acquire an HMQC spectrum requiring 8 min (8 transients/16×2 hypercomplex files). The F_2 (proton frequency domain) projection of the spectrum is shown in Figure 1A; the signal-to-noise (s/n) ratio was 10.8:1. A sample of 180 μg of **1** was also dissolved in 620 μl of solvent and HMQC spectrum was acquired using a conventional 5 mm inverse probe. The



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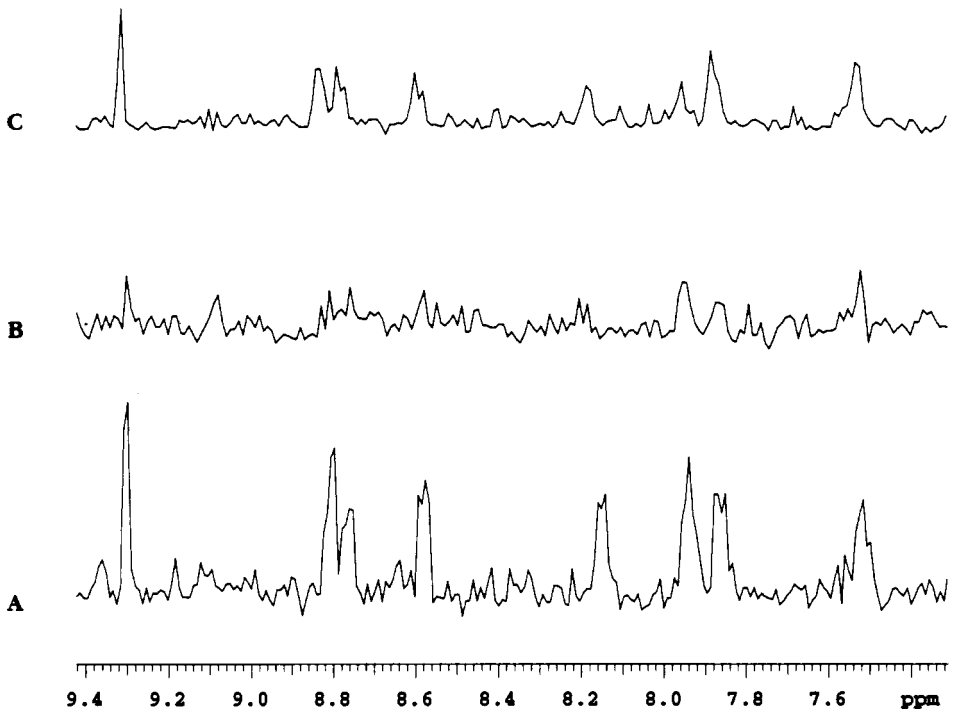

FIGURE 1. Projections of inverse-detected HMQC (6) spectra of cryptolepine HCl [**1**] dissolved in 99.96% DMSO-d_6. (A) F_2 (proton frequency domain) projection of the HMQC spectrum acquired using 200 μg of **1** dissolved in 200 μl of solvent in 8 min. (8 transients/16×2 hypercomplex files) on a Varian Unity 500 spectrometer equipped with a Z·SPEC® MID500 micro inverse probe supplied by Nalorac Cryogenics Corp. The data were processed using Gaussian multiplication prior to the first Fourier transform and cosine multiplication prior to the second and were zero-filled to 2K×128 points. The signal-to-noise (s/n) ratio of the projection was 10.8:1 (B) Comparison F_2 projection from an HMQC spectrum acquired on 180 μg of **1** dissolved in 620 μl of solvent using a Z·SPEC® 5 mm TID500 probe in 8 transients/16×2 hypercomplex files. The s/n ratio was 4.8:1 (C) Comparison F_2 projection from a 34 min acquisition using the probe and sample from trace B. The data were acquired in 34 min as 32 transients/16×2 hypercomplex files. The s/n ratio was 13.4:1. The projected data reflect essentially a twofold improvement in s/n in going from a conventional 5 mm inverse probe to a micro probe for the same sample. The sensitivity advantage gained with the micro probe corresponds to a fourfold time savings for experiments with equivalent s/n ratio projections.

projection of the HMQC spectrum recorded in the 5 mm probe in 9 min (8 transients/16×2 hypercomplex files) is shown in Figure 1B. The s/n ratio of the trace shown in Figure 1B was 4.8:1, essentially half that obtained with the microprobe in equivalent time. To attain an s/n ratio comparable to that obtained in 8 min with the microprobe, it was necessary to acquire data for 34 min (32 transients/16×2 hypercomplex files). The F_2 projection of the 34 min HMQC spectrum acquired in the 5 mm inverse probe is shown in Figure 1C; the s/n ratio was 13.4:1. The difference in the s/n ratios of the projected HMQC data acquired in the two probes corresponds to roughly a fourfold time advantage for the microprobe, corresponding roughly to a twofold increase in sensitivity per unit time with the microprobe.

At the level of 50 μg of **1** in 160 μl of solvent, HMQC data were obtained in 50 min (64 transients/16×2 hypercomplex files). More interesting, however, are the results obtainable with 12 μg of **1**. The spectrum recorded in 16 h (256 transients/16×2

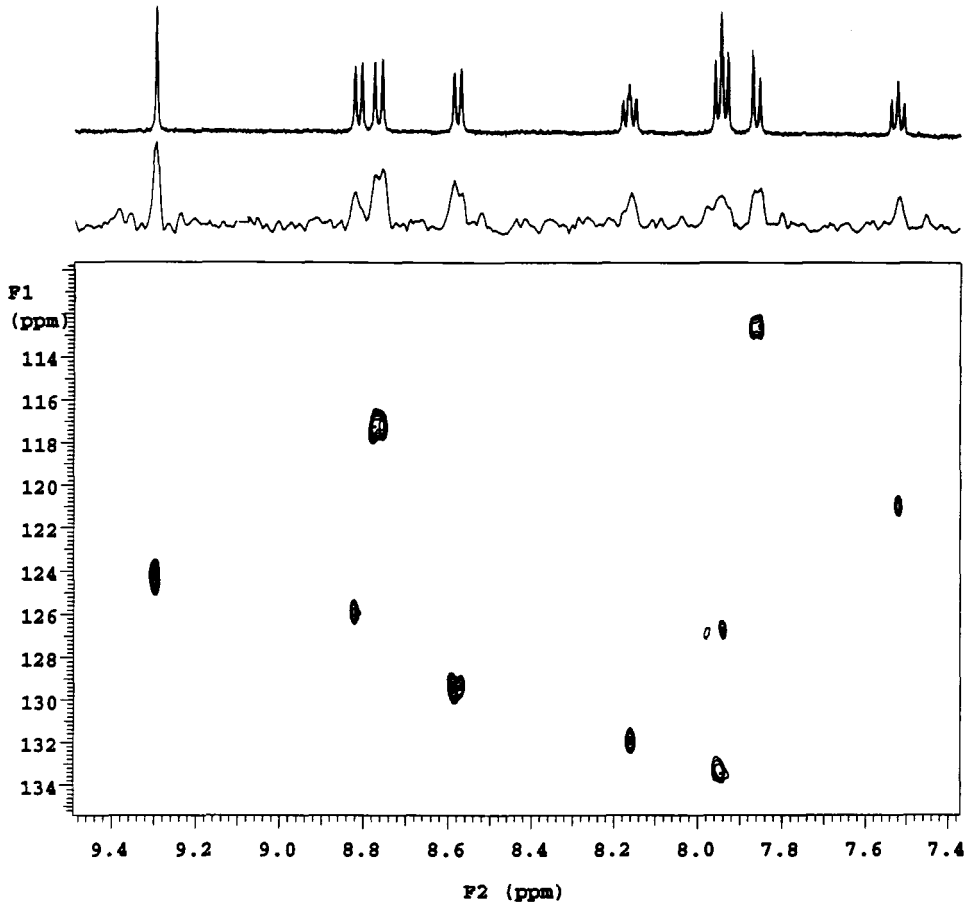

FIGURE 2. HMQC (6) spectrum recorded on 12 μg of cryptolepine HCl [**1**] dissolved in 145 μl DMSO-d_6. The data were recorded using 1536 points in F_2 and 24×2 hypercomplex pairs in F_1. Processing was identical to that used for the data shown in Figure 1. A total of 640 transients were accumulated per t_1 increment; total experiment time was 16 h. The proton reference spectrum plotted above the contour plot was recorded on the same sample in 256 transients. The F_2 projection is plotted between the reference spectrum and the contour plot.

hypercomplex files) on 12 μg of **1** is shown in Figure 2. The F_2 projection and a 256 transient conventional reference spectrum are plotted above the contour plot. The intensity of all of the responses is well above the noise floor of the spectrum and, in principal, the acquisition time could probably be reduced somewhat without the origin of any of the responses being in question.

As exciting as the prospect is of obtaining one-bond ($^1J_{CH}$) heteronuclear shift correlations on a 12 μg sample, this capability will still not allow the deduction, in most cases, of a molecular structure. A more germane question is the feasibility of acquiring HMBC spectra at this level. Although somewhat more time-consuming, an excellent HMBC spectrum shown in Figure 3 was recorded on a 35 μg sample of **1** in 21 h. Long-range connectivity information contained in this HMBC spectrum allows the unequivocal confirmation of the structure of cryptolepine in a fashion analogous to that recently used (12) in the assignment of the ^{1}H- and ^{13}C-nmr spectra of **1**.

In conclusion, the ability to acquire high quality HMQC and HMBC spectra on very

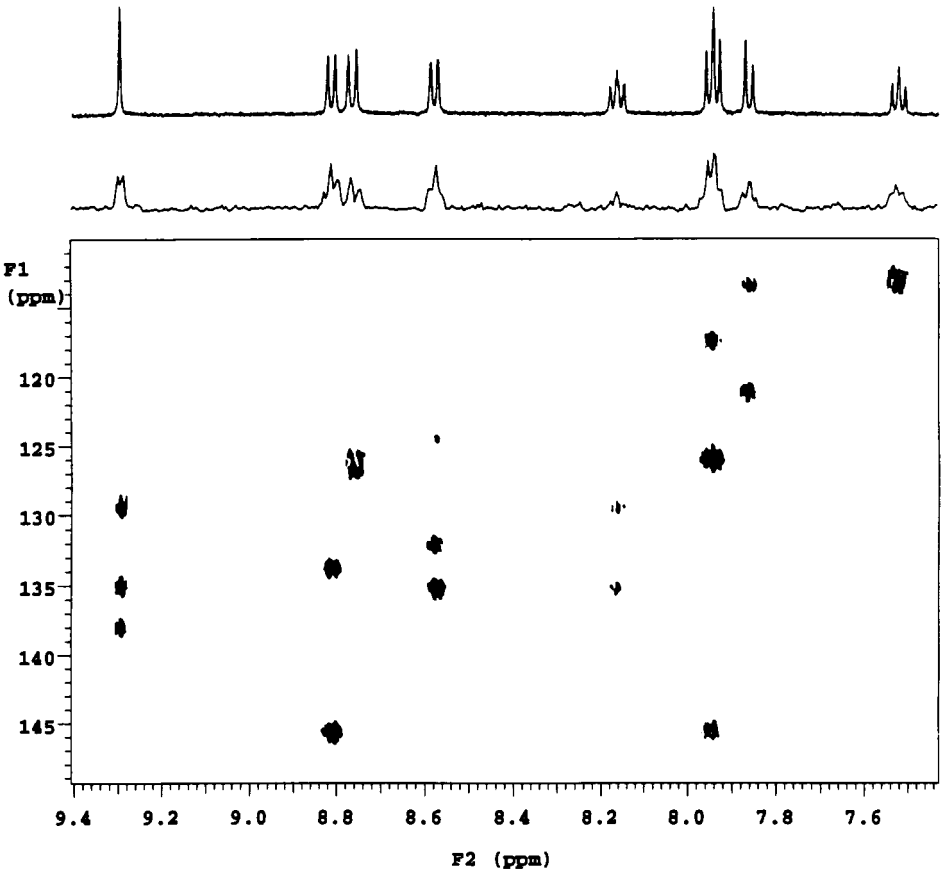

FIGURE 3. HMBC(10) spectrum recorded on 35 μg of cryptolepine HCl [**1**] dissolved in 145 μl of DMSO-d_6. The long-range delay was optimized for 63 msec (8 Hz); the data were recorded using 1536 data points in F_2 and 64×2 hypercomplex pairs in F_1. Data were processed using a phase-shifted Gaussian function prior to the first Fourier transform and cosine multiplication prior to the second. The data were zero-filled to 4K×256 points during processing. A total of 384 transients were accumulated per t_1 increment giving a total acquisition time of 21 h.

small samples of material, when coupled with proton-proton connectivities from a COSY spectrum which are easily attainable at this level (18 min on 12 μg using the microprobe), affords the natural product chemist a significant enhancement in the power of the techniques available to elucidate structures. Hopefully, these techniques will make it possible to determine the structures of potentially interesting minor natural product constituents that have been heretofore ignored because of the considerable difficulty in obtaining sufficient quantities to acquire useful spectal data.

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