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Differentiation of HMBC Two- and Three-Bond Correlations: A Method to Simplify the Structure Determination of Natural Products

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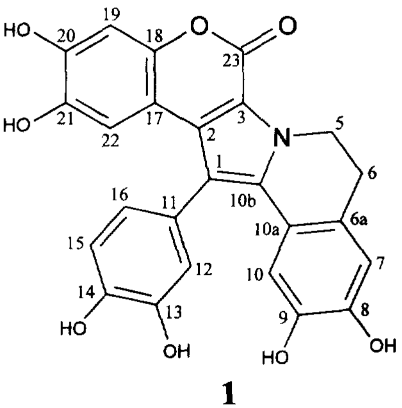
Abstract: The structure elucidation of natural products, today, relies heavily on the application of proton-detected heteronuclear NMR experiments. Perhaps the most useful of these methods is the HMBC experiment, which provides correlations between protons and carbons over two and three bonds. The application of the HMBC method for the direct translation of H,C correlations to yield bonding information is limited, however, by the fact that it does not distinguish between $^2J_{CH}$ and $^3J_{CH}$ correlations. Reported here is an application of the recently described 1,1-ADEQUATE experiment that yields only two bond H,C connectivities in H-C-C moieties and therefore allows the differentiation of HMBC two- and three-bond correlations. The method is demonstrated on a 14 mg sample of a new marine natural product, 5,6-dihydro lamellarin H (1).

The HMBC NMR experiment[1] has proven to be an extremely useful tool for the total structure elucidation of complex natural products.[2] The method allows the assignment of structural fragments through correlations of protons and carbons, via H,C-coupling constants, through one, two and three bonds. Direct correlations ($^1J_{CH}$) are usually suppressed by a low-pass J filter[3] leading to observation of only $^2J_{CH}$ and $^3J_{CH}$ correlations. Recently, the HMBC technique was modified by the introduction of pulsed-field gradients[4], which improved the interpretation of HMBC spectra by suppressing center signals (t_1-noise), which originate from protons bound to ^{12}C.[5] The direct translation of the connectivities observed in the HMBC into the bonding network is hampered by the fact that $^2J_{CH}$ and $^3J_{CH}$ correlations can not be distinguished.

In this communication, we introduce a new method that differentiates between $^2J_{CH}$ and $^3J_{CH}$ HMBC correlations. The new method yields only "pseudo $^2J_{CH}$ correlations" by combination of $^1J_{CH}$ and $^1J_{CC}$ transfer steps. Less sensitive versions of this experiment, the INEPT-INADEQUATE[6], the DEPT-INADEQUATE[7], the C-Relayed H,C-COSY[8] and the proton-detected INEPT-INADEQUATE[9] have been described. The experiment presented here is a refocused version of the 1,1-ADEQUATE[10] experiment. It evolves the chemical shift of C_2 during t_1 and detects the chemical shift of H_1 during t_2 for a H_1-C_1-C_2 moiety in contrast to the normal 1,1-ADEQUATE experiment or other INADEQUATE variants, where double quantum chemical



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shift of C_1 and C_2 evolves during t_1. Thus, protonated carbons are exclusively correlated with their directly attached carbons in the ω_1-refocused 1,1-ADEQUATE. This correlation is exclusive because of the large difference in size of ${}^1J_{CH}$ compared to ${}^nJ_{CH}$, and also ${}^1J_{CC}$ compared to ${}^nJ_{CC}$ coupling constants. The ω_1-refocused 1,1-ADEQUATE therefore shows only pseudo ${}^2J_{CH}$ correlations and has the advantage of being observed in an identical two dimensional format as are HMBC data. By overlaying both spectra, ${}^2J_{CH}$ and ${}^3J_{CH}$ correlations can be differentiated.

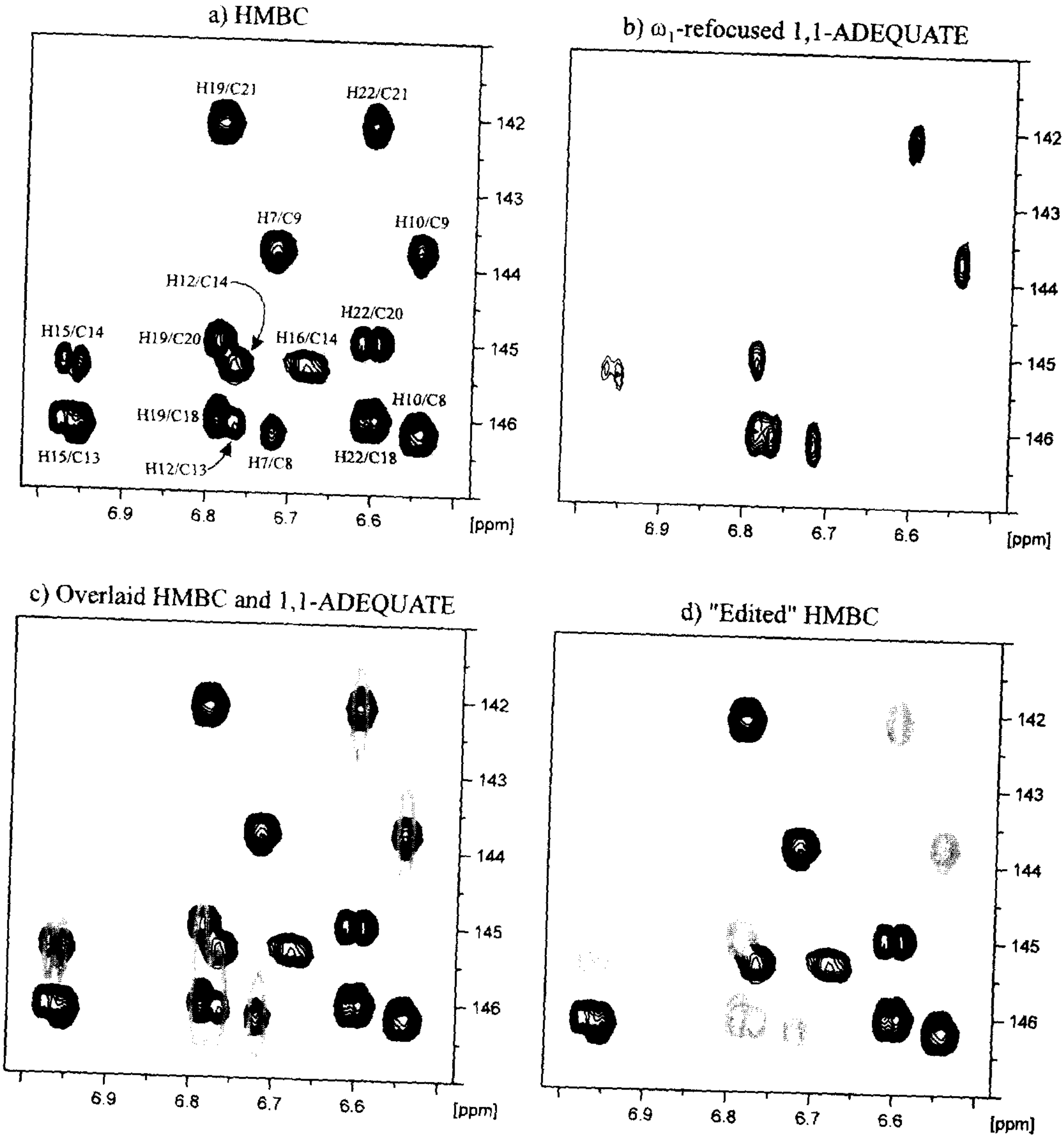
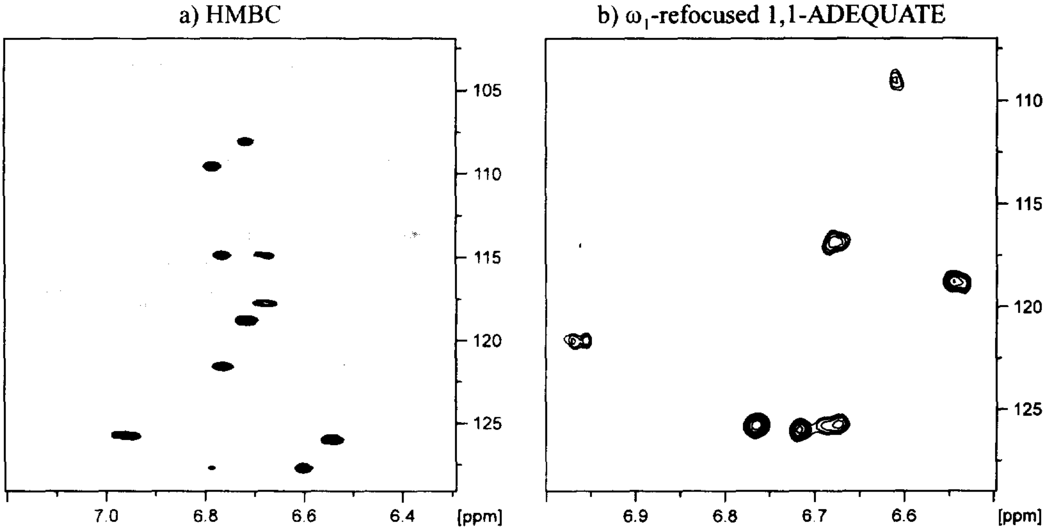


Fig. 1: *An expansion of the 141 to 147 ppm region of the HMBC spectrum of **1** (a), and the ω_1-refocused 1,1-ADEQUATE spectrum of **1** (b), illustrating the same region, are shown. The chemical shifts in the ω_1 frequency domain of the latter experiment are ${}^{13}C$ single quantum frequencies, and therefore the two spectra can be overlaid as*

shown in (c). The peaks of the 1,1-ADEQUATE spectrum are shown only with one contour line in grey; the resolution in ω_1 of this experiment was decreased for effective comparison. The same part of the HMBC is shown again (d), in which the 1,1-ADEQUATE data is used to edit the HMBC spectrum with respect to ${}^2J_{CH}$ and ${}^3J_{CH}$ correlations. Correlations from ${}^2J_{CH}$ couplings are plotted in grey while correlations from ${}^3J_{CH}$ couplings are shown in black.



a) HMBC

b) ω_1-refocused 1,1-ADEQUATE

c) Overlaid HMBC and 1,1-ADEQUATE

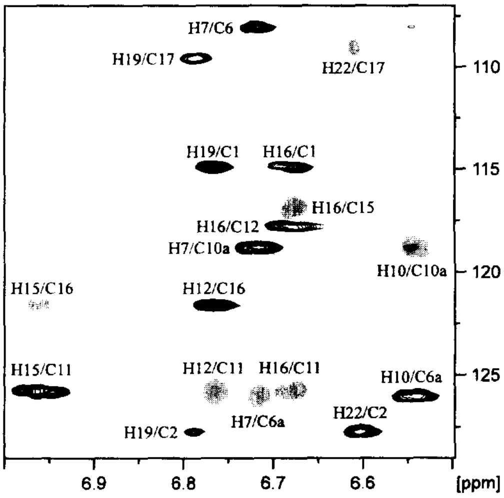


Fig. 2: *An expansion of the 102 to 129 ppm region of the HMBC spectrum of 1 (a). In (a), the direct correlations (${}^1J_{CH}$) were not suppressed and are shown in grey. An expansion of the 107 to 129 ppm region showing the w_1-refocused 1,1-ADEQUATE result with 1. For a more effective comparison of the two spectra, the HMBC spectrum and 1,1-ADEQUATE spectrum are overlaid in (c). For a better overview, the cross peaks resulting from direct correlations (${}^1J_{CH}$) are not shown. The peaks of the 1,1-ADEQUATE spectrum are shown in grey. Six ${}^2J_{CH}$ correlations (grey peaks superimposed on HMBC peaks) were not observed in the HMBC presumably due to very small ${}^2J_{CH}$ couplings. The 1,1-ADEQUATE data therefore complements the information derived from the HMBC experiment.*

To demonstrate the method, we utilized the polycyclic aromatic natural product **1**, isolated from a marine ascidian of the genus *Didemnum*. An expansion of the HMBC spectrum of **1**, showing the region 141 to 147 ppm, is shown in Figure 1a, while the ω_1-refocused 1,1-ADEQUATE experiment, showing the identical region, is shown in Figure 1b. For a better comparison of the two spectra, the HMBC and the 1,1-ADEQUATE two dimensional data are overlaid as shown in Figure

1c. Overlaying the spectra allows ${}^2J_{CH}$ and ${}^3J_{CH}$ correlations to be distinguished by their different grey shading ("Edited" HMBC, Figure 1d). Seven of fifteen correlations in this expansion of the HMBC spectrum were identified as ${}^2J_{CH}$ correlations.

In Figure 2, expansions of the two dimensional HMBC spectrum (Fig. 2a) and of the ω_1-refocused 1,1-ADEQUATE spectrum of **1** (Fig. 2b), showing the 102 (107) to 129 ppm region, are illustrated. Figure 2c shows the overlay of these two dimensional spectra. In this region, most of the ${}^2J_{CH}$ correlations are missing in the HMBC spectrum. Thus, the 1,1-ADEQUATE data also complements the HMBC results by yielding ${}^2J_{CH}$ correlations that are notoriously weak in olefinic and aromatic systems.

In summary, the ω_1-refocused 1,1-ADEQUATE is an useful method to accentuate ${}^2J_{CH}$ correlations leading, in combination with typical HMBC spectra, to a comprehensive identification of ${}^2J_{CH}$ and ${}^3J_{CH}$ correlations. The distinction between ${}^2J_{CH}$ and ${}^3J_{CH}$ connectivities allows the assignment of complex structures to proceed with significantly greater ease and reliability.

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