

ANALYSIS OF THE ^1H -NMR SPECTRUM OF THE ANTITUMOR AGENT MITINDOMIDE AND DERIVATIVES.
AN UNUSUAL EXAMPLE OF NONEQUIVALENT VICINAL OLEFINIC PROTONS

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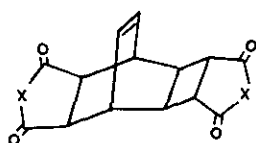
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Abstract - The ^1H -nmr spectrum of the antitumor agent mitindomide (**1b**) in $\text{DMSO}-d_6$ solution has been completely analyzed by the use of homonuclear decoupling, NOE effects and specific deuterium labeling. A derivative of mitindomide, in which asymmetric groups were attached to the imide nitrogen (**2c**), showed magnetically nonequivalent diastereiotopic olefinic protons.

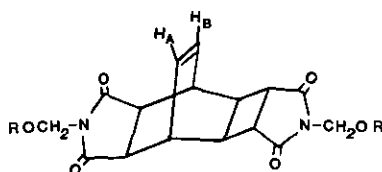
INTRODUCTION

The photochemical reaction between benzene and maleic anhydride was first reported in 1959¹ and the structure of the adduct was assigned as **1a** largely by chemical means^{2,3}. Maleimide was



1a X = O

1b X = NH



2a R = H

2b R = COCH_3

2c R = $\text{COCH}(\text{CH}_2\phi)\text{NH}_3^+\text{Cl}^-$

2d R = $\text{COCH}(\text{CH}_2\phi)\text{NH-tBOC}$

also shown^{4,5} to photochemically add to benzene to produce a compound (**1b**, mitindomide) whose structure was assumed based on analogy with **1a**. The structure of a derivative of mitindomide, compound **2b**⁶, as well as an hydrolysis product⁷, have been determined by X-ray crystallography. Interest in the bisimide **1b** was stimulated by the observation of strong and reproducible antitumor activity in several test systems⁸. Our work in this area has centered on the synthesis of congeners and prodrugs of **1b** with improved properties such as solubility, selectivity, potency, activity, and toxicity.⁹

Only very little ^1H -nmr data has been reported on **1b** and compounds related to it. The most complete data reported⁷ was determined at 100 MHz in DMSO- D_6 solution and did not report any assignments. In fact, some absorptions are reported incorrectly. Thus a broad singlet at 3.30 ppm is not part of the sample, but rather the typical water absorption, normally seen in DMSO- D_6 . In addition, the signals reported as two doublets at 6.30 and 6.34 ppm, in fact, obviously represent the olefinic protons which are magnetically equivalent (isochronic). Our goal in this publication is to report a complete assignment of the ^1H -nmr spectrum of **1b** and to illustrate what we believe is the first example of the nonequivalence of diastereotopic vicinal olefinic protons in derivative **2c**.

RESULTS AND DISCUSSION

The 300 MHz ^1H -nmr (DMSO- D_6) spectrum of mitindomide (**1b**) shows a broad singlet at δ 11.2 (imide N-H), a six line pattern at δ 6.30 (H_A') and a series of more and less broadened "singlets" at δ 3.08, 2.77, 2.57, and 2.54. The pattern for the olefinic protons δ 6.30 was simulated using the computer program RACON¹⁰, by a trial and error process starting with normal values for cis olefinic, vincinal, and allylic coupling constants. The comparison of calculated and observed spectra are shown in Figure 1. The calculated coupling constants are $J_{\text{AA}'} = 8.6$, $J_{\text{AC}} = 6.1$, and

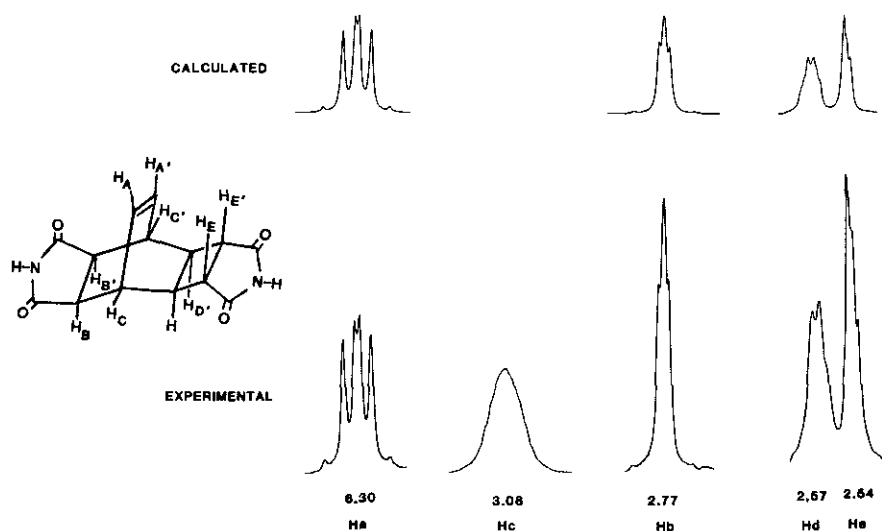


Figure 1. Calculated and Experimental 300 MHz ^1H -nmr Spectrum of Mitindomide in DMSO- D_6 .

$J_{\text{AC}}' = 1.6$ Hz. Although the absorptions for the other protons appear normally as broadened "singlets," under the highest resolution, all of these are actually complex signals. These are shown in Figure 1, as well as their assignments, which will now be discussed. Decoupling of the olefinic protons at δ 6.3 showed a small but significant increase in the height only of the

absorbance at δ 3.08, confirming this as H_C . Decoupling at δ 3.08 (H_C) showed the expected collapse in the signal at δ 6.30 (H_A).

By irradiation of the absorbance at δ 6.30 (H_A) a strong NOE enhancement was observed for the absorbances at δ 3.08 and 2.54, with no enhancement at δ 2.77 and 2.57. Inspection of models, as well as X-ray data, clearly show that proton H_E is quite close to H_A and thus the signal at δ 2.54 is assigned to H_E . This leaves only the peaks at δ 2.77 and 2.57 for assignment to either H_B or H_D . This was accomplished chemically by repeating the photochemical reaction using benzene- D_6 and maleimide. The mitinomide- D_6 produced would be expected to have deuterium in place of protons H_A , $H_{A'}$, H_C , $H_{C'}$, H_D and $H_{D'}$. The 1H -nmr spectrum of this material showed absorbances only at δ 11.2, 2.77 and 2.54, confirming the signals at δ 2.77 as H_B and δ 2.57 as H_D .

The absorbance at 3.08 (H_C) is very broad, $W_{1/2h} = 10$ Hz, and reveals no fine structure. The signal at 2.77 (H_B) is actually a five line pattern, with the outer two lines of very low intensity. Simulation with $J_{BB'} = 8.0$ and $J_{BC} = 3.0$ Hz gives good agreement as shown in Figure 1. The absorbances at δ 2.57 and 2.54 are more complex and difficult to analyze. Because many lines are not resolved, accurate coupling constants cannot be obtained. However, simulation with $J_{DD'} = J_{EE'} = 4.5$, $J_{ED} = 1.8$, $J_{DC} = 3.5$ Hz, and all other J 's equal to zero gives a reasonable match with experimental spectrum (see Figure 1). All of the chemical shift and coupling constant data are summarized in Table 1.

Table 1. Chemical Shift and Coupling Constant

Assignments for Mitinomide (1b)		
Proton	Chemical Shift-ppm	Coupling Constants-Hz
imide N-H	11.02	$J_{AA'} = 8.6$ $J_{AC'} = 1.6$
$H_A, H_{A'}$	6.30	$J_{BB'} = 8.0$ $J_{BC} = 3.0$
$H_B, H_{B'}$	2.77	$J_{DD'} = J_{EE'} = 4.5$
$H_C, H_{C'}$	3.08	$J_{ED} = 1.8$ $J_{CD} = 3.5$
$H_D, H_{D'}$	2.57	$J_{AC} = 6.1$
$H_E, H_{E'}$	2.54	ALL OTHER $J = 0$

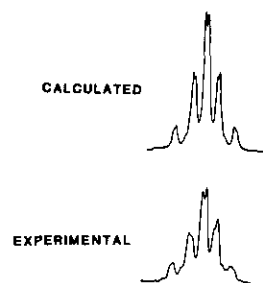


Figure 2. Calculated and Experimental 300 MHz 1H -nmr Spectrum of Compound 2c in D_2O

In connection with our work on the synthesis of prodrugs of 1b, compound 2c was made (see experimental) and its 300 MHz 1H -nmr spectrum determined in D_2O . The 1H -nmr spectrum of 2c clearly shows that all four methylene groups contain magnetically nonequivalent protons that can be readily analyzed. It has long been recognized that geminal protons or groups near asymmetric centers are diastereotopic and often show a measurable chemical shift difference (anisochronic or nonequivalent). This subject has received a number of excellent reviews.^{11,12} This same concept

applies to vicinally displaced atoms or groups, but has received little attention. A review of the literature finds only one case, the epoxide ring protons of scopolamine, in which vicinal nonequivalence of this type was observed.¹³ Review articles^{11,14} mention that diastereotopic vicinal protons on a double bond should show this effect, but no examples have been reported. However, the olefinic protons in **2c**, H_A and H_B , show a pattern which is quite different than the parent compound. This is shown in Figure 2. Attempts to simulate this pattern by changing the coupling constant assignments made for mitinomide were not successful, even when large changes were tried. Since no substantial conformational changes would be expected in this rigid system, some other explanation of the ¹H-nmr spectrum must be sought. The observed spectrum can be readily simulated, however, by using the same coupling constants as mitindomide, and making the chemical shift difference between H_A and H_B , $\Delta\nu_{AB}$, equal to 10.5 Hz (0.035 ppm), as shown in Figure 2. As far as we are aware, this is the first example of observed magnetic nonequivalence of diastereotopic vicinal olefinic protons. Although the effect not large, it is certainly long range in nature and indicates the difficulty in predicting the magnitude of these differences. For example, the -CH₂- groups of **2c** immediately adjacent to the asymmetric center shows $\Delta\nu$ values of 25.8 and 8.9 Hz. The -CH₂- groups that are four bonds removed (adjacent to nitrogen), show $\Delta\nu$ values of 71.6 and 73.7 Hz. Since the olefinic protons are at least eight bonds removed, distance alone is not the determining factor. As has been pointed out¹² no general theory exists which can predict the magnitude of these chemical shift differences.

EXPERIMENTAL

Compounds **1b** and **2a** were made as previously described.⁹ All spectra were acquired using a Bruker WP-300 spectrometer, using the Aspect 2000 data system and the program DISMNRP. All chemical shift values are relative to the solvent the sample was determined in, DMSO $\approx$ 2.49 and water = 4.65 ppm. The computer program RACOON was used, on an IBM PC, to simulate all ¹H-nmr spectra.

Synthesis of Compound 2c. To a solution of 11.2 g (0.0421 mole) of N- α -t-BOC-L-phenylalanine (Chemical Dynamics) in 120 ml of dry distilled DMF was added 6.83 g (0.0421 mole) of carbonyl dimidazole. After 1 h, 3.50 g (0.0105 mole) of **2a** dissolved in 100 ml of DMF, was added and the solution allowed to stand for 1.5 h. After evaporation of the solvent, 100 ml of cold water was added and the mixture stirred. The solid was filtered, washed with water, and then dissolved in CH₂Cl₂. The solution was extracted repeatedly with NaHCO₃ and then dried. Removal of the solvent gave 7.0 g (81%) of a red-orange foam which was chromatographed on silica gel. Elution with CHCl₃ gave 4.3 g (50%) of **2d**, which was a white foam. TLC and ¹H-nmr indicated this material was pure. A sample, 3.20 g (0.00387 mole), of **2d** was dissolved in 40 ml of 25% v/v trifluoroacetic acid in CH₂Cl₂. After 0.5 h the solvent was removed, and the residual material dissolved in water

and freeze dried. The solid that was obtained was dissolved in 25 ml of THF and excess 2.0N HCl in dioxane added. The precipitate was collected and dried to yield 1.82 g (62.0%) of **2c** which showed mp ca 170 °C (dec), $[\alpha]_D^{25} = 9.3^\circ$, 300 MHz $^1\text{H-NMR}(\text{D}_2\text{O})$ δ 7.4 - 7.1 (aromatic), 6.27 (olefinic, see text), 5.62 and 5.39 (N-CH₂-O, J = 10.6 Hz), 5.57 and 5.33 (N-CH₂O, J = 10.6 Hz), 4.39 and 4.36 (CH, J = 6.8 Hz), 3.32 (bs), 3.17 and 3.08 (CH₂-C, J = 14.5 and 6.8 Hz), 3.14 and 3.11 (CH₂-C, J = 14.5 and 6.8 Hz), 2.94 (bs), 2.78 (bs), and 2.59 (bs). Anal. C₃₄H₃₆N₄O₈Cl₂·4.2 H₂O, C, H, N, Cl.

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