# THE $^{13}$C-NMR SPECTRUM OF MITOMYCIN C: REASSIGNMENT

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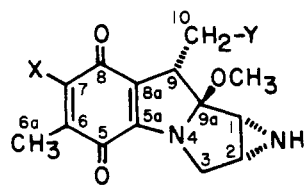
ABSTRACT.—The $^{13}$C-nmr spectrum of the antitumor antibiotic mitomycin C was first reported in 1974 by Lown and Begleiter who provided reasonable, but not unambiguous, assignments. The need for unambiguous $^{13}$C-nmr assignments of mitomycin C for biosynthetic studies and mode of action studies prompted our reinvestigation. The use of various proton decoupling techniques, $^{15}$N-labeling, and spectral comparisons with decarbamoylmitomycin C led to unambiguous assignments for all of the resonances in the mitomycin C $^{13}$C-nmr spectrum and, thus, to a revision of the published assignments for C-5, C-6, C-8, C-8a, and C-9a.

Mitomycin C (**1**) is a member of a group of closely related antitumor antibiotics that are produced by various species of *Streptomyces* (1). Mitomycin C is currently in clinical use as an anticancer agent (2).

In preparation for continued biosynthetic and mode of action studies on mitomycins (3,4), we found it necessary to re-examine the assignments previously published by Lown and Begleiter (5) for the $^{13}$C-nmr spectrum of mitomycin C. The work of these authors relied on proton-noise-decoupled and off-resonance-decoupled spectra, as well as on chemical shift comparisons with model compounds, and yielded reasonable, but not unambiguous, assignments. However the high degree of unsaturation and the presence of a pseudo-center of symmetry in the quinone ring make the carbon assignments difficult. In the present work, the assignment of the $^{13}$C-nmr spectrum of mitomycin C was investigated by several means including: $^{1}$H-broadband decoupling, gated $^{1}$H-decoupling, single frequency off-resonance decoupling (SFORD), low-power selective proton decoupling, $^{15}$N-labeling, and spectral comparisons with decarbamoylmitomycin C (**2**).

## EXPERIMENTAL

$^{13}$C-nmr spectra of mitomycin C (0.2 M in 2.3 ml of pyridine-$d_5$) were measured at 50.3 MHz on either a Varian XL-200 spectrometer or a Nicolet NT-200 instrument, which were operated in FT mode: pulse angle = 45°, acquisition time = 1.5 sec, repetition time = 3.0 sec, sweep width = ± 5500 Hz, 32 K data sets. Gated (decoupler off only during acquisition) and continuous broadband $^{1}$H-decoupled spectra were obtained by square wave modulation of the decoupler frequency. SFORD experiments were conducted with the proton decoupler set at −2.5 ppm and $\gamma_H H_2/2\pi$ = 2.85 KHz. Low power selective pro-




| | X | Y |
|---|---|---|
| 1 | $NH_2$ | $OC(=O)NH_2$ |
| 2 | $NH_2$ | OH |
| 3 | $^{15}NH_2$ | $OC(=O)NH_2$ |
| 4 | $OCH_3$ | $OC(=O)NH_2$ |

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ton decoupling was accomplished with a decoupler power which gave $\gamma_H H_2/2\pi = 116$ Hz. The $^1$H-nmr spectrum of the same sample of mitomycin C used for the $^{13}$C-nmr experiments was measured on a Varian FT-80 instrument: pulse angle = 60°, acquisition time = 4.1 sec, repetition time = 7.1 sec, sweep width = 1000 Hz, 8 K data sets. All chemical shifts are referenced to internal TMS.

($C_7$-$^{15}NH_2$)-Mitomycin C (**3**) was prepared from mitomycin A (**4**), which was adsorbed on silica gel and then slurried in a mixture of hexanes and exposed to $^{15}$N-ammonia for 12 h (6). Decarbamoylmitomycin C was prepared by the method of Kinoshita *et al.* (7) through reaction of mitomycin C with sodium methoxide. Both compounds exhibited the expected spectral characteristics.

## RESULTS AND DISCUSSION

The results obtained in the SFORD experiment (table 1) in conjunction with the $^1$H-nmr assignments for mitomycin C[2] established by Lown and Begleiter (5) and the results obtained through analysis of one bond and long-range $^1$H-$^{13}$C couplings, yield assignments for the protonated carbons of **1** which conform to the published assignments (5). The nonprotonated carbon signals, which comprise the remainder of the spectrum, are found in three spectral regions: three peaks at 104-111 ppm, three peaks at 149-159 ppm, and two signals at 176-179 ppm.

TABLE 1 50.3 MHz $^{13}$C-nmr data for mitomycin C and related compounds.

| Assignments[a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| New | Old | ppm[a] | Height[a] | $^1J_{C\text{-}H}$[b] | $^{2,3}J_{C\text{-}H}$[b] | $J^r$[c] | $J^r/^1J$ × 100 | **3**[d] ppm | **2**[e] ppm | **2** Height |
| 5 | 8 | 178.3 | 21 | s | 3, q | — | — | 179.0 | 178.5 | 15 |
| 8 | 5 | 176.7 | 38 | s | 5, t | — | — | 177.1 | 177.3 | 32 |
| 10a | 10a | 158.1 | 81 | s | s | — | — | 159.6 | — | — |
| 5a | 5a | 156.1 | 32 | s | s | — | — | 157.4 | 156.0 | 22 |
| 7 | 7 | 149.6 | nm[f] | s | nm | — | — | 151.6[g] | 149.8 | nm |
| 8a | 9a | 110.7 | 65 | s | 7, d | — | — | 110.6 | 113.0 | 27 |
| 9a | 6,8a | 106.8 | 44 | s | m | — | — | 107.3 | 107.6 | 22 |
| 6 | 6,8a | 104.3 | 80 | s | 5, q | — | — | 104.9 | 104.1 | 53 |
| 10 | 10 | 62.5 | 100 | 150, t | 8, d | 81 | 54 | 63.1 | 61.3 | 91 |
| 3 | 3 | 50.6 | 87 | 146, t | m | 65 | 45 | 50.8 | 50.7 | 91 |
| OMe | OMe | 49.5 | 89 | 142, q | s | 60 | 42 | nm[f] | 49.6 | 71 |
| 9 | 9 | 44.2 | 79 | 134, d | s | 64 | 48 | 44.4 | 47.9 | 100 |
| 1 | 1 | 36.7 | 65 | 181, d | m | 76 | 42 | 37.5 | 36.5 | 56 |
| 2 | 2 | 32.6 | 57 | 182, d | m | 68 | 37 | 33.5 | 33.5 | 42 |
| 6a | 6a | 8.8 | 98 | 127, q | s | 40 | 31 | 8.1 | 8.8 | 86 |

All J values are in Hertz.
[a]$^1$H-decoupled data for mitomycin C (**1**).
[b]Gated decoupling experiment for (**1**).
[c]SFORD experiment for (**1**).
[d]33 mg in 2.3 ml of methanol-$d_4$.
[e]48 mg in 2.3 ml of pyridine-$d_5$.
[f]Partially obscured by solvent, nm = not measured.
[g]Doublet, $J$ = 17 Hz.

The three resonances in the high-field aromatic region are expected to be due to C-6, C-8a, and C-9a on the basis of their chemical shifts. Their long-range couplings, as observed in the gated decoupling experiment, initially suggested the new assignments shown in table 1; however, these new assignments implied relatively large two-bond $^1$H-$^{13}$C couplings and small or nonexistent three-bond couplings. Low-power selective proton decoupling (table 2) nonetheless confirmed these assignments. Thus, irradiation of the quinone methyl protons caused a 54% decrease in the line width at one-half

[2]Mitomycin C 80 MHz $^1$H-nmr, 0.2 M in pyridine-$d5$: 2.03 ppm, s, $C_6CH_3$; 2.75, m, $C_2H$; 3.11, d, $C_1H$; 3.22, s, $OCH_3$; 3.60, dd, $C_3H$; 4.02, dd, $C_9H$; 4.56, d, $C_3H'$; 5.08, t(dd), $C_{10}H$; 5.45, dd, $C_{10}H'$.

TABLE 2. Low power selective $^1$H-decoupling data for mitomycin C.

| $^1$H-decoupler | | $\Delta\nu$½ in Hz for $^{13}$C-nmr signals at (ppm) | | | | |
|---|---|---|---|---|---|---|
| ppm | $^1$H-assignment | 104.3 | 106.8 | 110.7 | 176.7 | 178.3 |
| 2.03 | $C_6CH_3$ | 10 | 25 | 11 | 14 | 6 |
| 4.02 | $C_9H$ | 20 | 28 | 4.5 | 11 | 13 |
| −15 | Control | 22 | 23 | 11 | 12 | 15 |

height ($\Delta\nu$½) for the 104.3 ppm signal, and the peak at 178.3 ppm narrowed by 60%. In a similar experiment on decoupling the C-9 proton, the 110.7 ppm peak collapsed to give a $\Delta\nu$½ value that was 40% of the control value due to the abolition of long range coupling.

The three signals in the low-field aromatic region arise from C-5a, C-7, and C-10a (carbamate carbonyl), as implicated by their chemical shifts. The resonance at 149.6 ppm was shown to be due to C-7 by the observation of a 17 Hz $^{15}$N-$^{13}$C coupling in the $^1$H-decoupled $^{13}$C-nmr spectrum of **3**. No longer-range $^{15}$N-$^{13}$C couplings were observed. The other two peaks in this region display no long-range $^1$H-$^{13}$C couplings, but there is a great disparity in their peak heights (*ca.* 2.5:1). The $^1$H-decoupled spectrum of decarbamoylmitomycin C (**2**), which was obtained under the same experimental conditions as for mitomycin C and which shows great overall similarity with the spectrum of the parent compound, retains only the smaller peak at almost the same chemical shift as the small peak in the mitomycin C spectrum. These experiments confirmed the assignments for C-5a, C-7, and C-10a given in table 1.

The quinone carbonyl signals at 176.7 and 178.3 ppm are reassigned on the basis of their long-range $^1$H-$^{13}$C coupling patterns and the aforementioned selective decoupling of $C_6CH_3$.

The data presented above lead to an unambiguous assignment of the $^{13}$C-nmr spectrum of mitomycin C and thus to a revision of the previous assignment (5) for C-5, C-6, C-8, C-8a, and C-9a (8).

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