Reassignment of the ^{13}C NMR spectrum of minomycin

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(received 08 Apr 03; accepted 23 Oct 03; published on the web 31 Oct 03)

Abstract

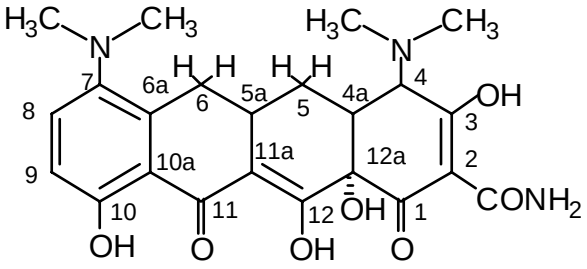
^{1}H and ^{13}C NMR spectra of minomycin were determined and a full assignment was carried out with the aid of HMQC, HMBC and other techniques. It was shown that the previous assignment for ^{13}C-NMR signals required some correction.

Keywords: ^{13}C NMR, ^{1}H NMR, HMBC, HMQC, antibiotics, isotope shift

Introduction

As an extension of our synthetic and structural studies of organogermanium compounds[1], we were interested to study the changes of physiological activity induced in antibiotics by the introduction of a germanium-containing moiety.

The first antibiotic we chose for this purpose was minomycin (minocycline) hydrochloride (1). It is essential to have a full assignment of the ^{1}H and ^{13}C NMR spectra of **1** in order to elucidate the structure of the reaction products of germylation.



1

Figure 1

Two papers have so far been published on the NMR spectra of 1, one by Nilges *et al.*[2] on ^{1}H NMR, and another by Mazzola *et al.*[3] on ^{13}C NMR spectra. In the paper by Nilges *et al.*[2], as well

as considering the conventional chemical shifts, coupling constants were also used for the spectral assignment. Mazzola *et al*.[3] employed chemical shift considerations and coupled splitting patterns in their study, although no modern techniques, such as 2D NMR, were used. It occurred to us that it was worthwhile to attempt a re-examination of the NMR spectra of **1** with the aid of 2D and other techniques. In this paper we report the reassignment of the ^{13}C NMR spectrum of **1**.

Results and Discussion

^{1}H NMR spectra

Nilges *et al*. determined ^{1}H NMR spectra of **1** in CD_3OD and DMSO-d_6 in order to differentiate between exchangeable and non-exchangeable protons. For protons 4, 4a, 5a, 5', 5", 6'and 6", homonuclear spin decoupling was extensively employed to facilitate the assignment. Signals due to H4a and H5a were obscured by the strong peak of one of the $N(CH_3)_2$ moieties at δ 2.99. They identified these signals by comparing the spectrum of a related compound that does not contain this $N(CH_3)_2$ moiety. The assignment of the remaining aliphatic ring protons was essentially based on the Karplus-type relation. From these results, the authors concluded that 1 has the same basic conformation as other tetracyclines.

We confirmed their assignment using a systematic proton homonuclear decoupling experiment. Thus, we irradiated the CD_3OD solution of 1 at δ 1.63 (H5'), 2.19 (H6'), 2.9 (H4a and H5a), 3.41 (H6") and 4.26 (H4). For instance, by irradiating H5', a doublet for H4a collapsed into a singlet, and a multiplet at δ 2.9 became a singlet, which indicated that the low field multiplet centered at δ 2.21 is due to H5" rather than H6' thus confirming the assignment made by Nilges *et al*.[2]

We further confirmed the assignment by an H-H COSY experiment. The starting point of choice is the signal for H4 at δ 4.26, which is a doublet with very small splitting due to coupling with H4a. There are no other couplings involved, and hence it is easy to identify from the splitting pattern. This signal is correlated with the signal for H4a at δ 2.9, and the correlation can be extended to the signals for H5'(δ 1.48) and H5"(δ 2.21) to confirm the reported assignment.

Chemical shifts and coupling constants are summarized in Table 1.

Table 1. ^{1}H NMR Spectral Data for 1

chemical shifts (δ)	ref. 2 in CD_3OD	in DMSO-d_6	this study in CD_3OD	in DMSO-d_6
H4	4.06d		4.26	4.26
H4a	2.93dt		*ca*. 2.9m	*ca*. 2.9m
H5'	1.64dt		1.63dt	1.49
H5"	2.20ddd		2.21m	2.20
H5a	2.9-3.0		*ca.* 2.9m	*ca*. 2.9m

H6'	2.19dd		2.19m	2.20
H6"	3.42dd		3.41dd	3.40m
H8	6.81d		6.82d	6.82d
H9	7.44d		7.42d	7.42d
4-$N(CH_3)_2$	2.99s		2.83s	2.83s
7-$N(CH_3)_2$	2.62s		2.55s	2.55s
10-OH		11.28		11.27
12-OH		14.8		14.8
NH_2'		9.52		9.50
NH_2"		9.07		9.04

coupling constants(Hz)[a]	ref. 2	this study
$J_{4a,4}$	1.59	
$J_{4a,5'}$	13.65	
$J_{4a,5"}$	2.86	2.75
$J_{5a,5'}$	11.11	
$J_{5a,5"}$	5.08	4.91
$J_{5a,6'}$	13.34	
$J_{5a,6"}$	4.12	4.4
$J_{5',5"}$	13.65	13.63
$J_{6',6"}$	15.55	15.65
$J_{8,9}$	8.79	8.8

[a] Data in CD_3OD.

^{13}C NMR spectra

Mazzola *et al*[3] reported the assignment of the ^{13}C NMR spectrum of **1** based on a comparison of chemical shifts against other tetracyclones, the splitting pattern under a proton coupled condition and the multiplicity observed in SFORD (single frequency off resonance decoupling) experiments. In one case they also used spin-lattice relaxation times (T_1).

Although the multiplicity in SFORD experiments and the splitting pattern in proton coupled spectra will give useful information, comparisons of chemical shifts between similar compounds are not always unambiguous, particularly among compounds with many substituents.

For this reason, we undertook our own assignment. Altogether twenty-one signals were observed for twenty-three carbon nuclei in **1**, with two equivalent methyl groups in each dimethylamino moiety. In Table 2, twenty-one ^{13}C chemical shifts and their multiplicity as determined by DEPT (DEPT90 and DEPT135), are listed.

Table 2. Chemical shifts and multiplicity of ^{13}C peaks of minomycin (**1**)

Peak no	chemical shift (δ)

primary	5	42.4
	6	45.0
secondary	1	30.2
	3	35.0
tertiary	2	32.0
	4	35.2
	7	69.0
	12	116.5
	13	129.2
quaternary	8	74.1
	9	96.0
	10	109.2s
	11	116.2
	14	137.2
	15	142.1
	16	158.0
	17	172.0
	18	174.1
	19	188.0
	20	194.0
	21	194.1

Table 2 demonstrates that there are cases where many carbon nuclei with the same multiplicity have close chemical shift values. More advanced techniques are thus required for the unambiguous assignment of all carbon nuclei.

Firstly, we used HMQC (Heteronuclear Multiple Quantum Correlation)[4], by which the six correlations summarized in Table 3 were established. Peaks 7, 12 and 13 were readily assigned to C4, C8 and C9, respectively, based solely on chemical shifts and multiplicity. Peak 5 is automatically assigned to 4-N($\underline{C}H_3)_2$ since peak 6 is correlated with another N($\underline{C}H_3)_2$ moiety.

Table 3. Correlation established by HMQC in CD_3OD

^{1}H shift; δ (assignment)	^{13}C peak No; δ (assignment)
1.63 (H5')	3 (C5)
2.55 (7-N($C\underline{H}_3)_2$)	6 (7-N($\underline{C}H_3)_2$)
3.33 (H6")	1 (C6)
4.06 (H4)	7 (C4)
6.82 (H8)	12 (C8)
7.42 (H9)	13 (C9)

The assignment of ^{13}C peaks 2 and 4 (either C4a or C5a) by HMQC was difficult since the H4a and H5a peaks are nearly overlapping. The assignment of these two peaks, together with all

other quaternary carbon nuclei, can be achieved with the aid of HMBC (Heteronuclear Multiple Bond Correlation)[5]. Figure 2 is the HMBC spectrum of **1** in DMSO-d_6.

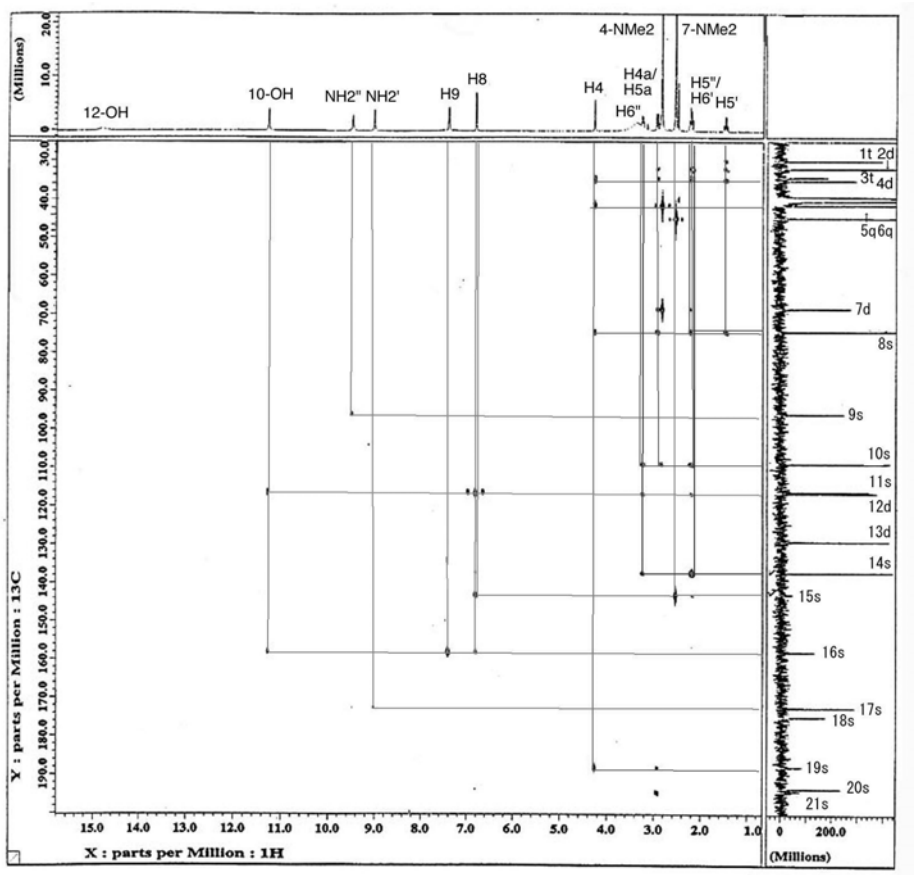


Figure 2. HMBC spectrum of **1**.

It is rather unfortunate for HMBC that the H4a and H5a peaks, and H5" and H6' peaks are overlapping, making the assignment rather ambiguous. However, with the aid of some chemical shift considerations, the assignment was carried out as given below.

(1) The H4 peak correlates with ^{13}C peak 4d but not with peak 2d; hence peak 4d can be assigned to C4a. Consequently, the remaining doublet ^{13}C peak 2d is assigned to C5a.

(2) The H4 peak correlated with peak 5q, one of the $N(CH_3)_2$ moieties. Hence peak 5q should be assigned to 4-$N(CH_3)_2$, to confirm the assignment based on HMQC.

(3) It is expected that C12a should resonate at a relatively high field since it is an sp^3 hybridized species. Peak 8 is highest among the remaining twelve singlets and, hence, is assigned to C12a, This assignment can be confirmed by correlation with H5 peaks.

(4) One of the NH_2 peaks (δ 9.50) correlates only with ^{13}C peak 9s, while another NH_2 peak (δ 9.04) correlates with ^{13}C peak 17s. From chemical shift considerations, ^{13}C peak 9s should be C2, and ^{13}C peak 17s should be due to -$\underline{C}ONH_2$.

(5) ^{13}C peak 16s correlates with H8, H9 and 10-OH peaks. Hence peak 16s can be assigned to C10.

(6) The 10-OH peak shows a correlation with ^{13}C peak 11 (there is little possibility of correlation with peak 12 (C8)) which should be either C10a or C11a. The latter possibility is excluded, given the considerable distance between them and, accordingly, ^{13}C peak 10 is assigned to C11a.

(7) This assignment was confirmed by the correlation of the 10s peak with H4a, H5a, H5 and H6.

(8) One interesting correlation is the one between the 7-N(C<u>H</u>$_3$)$_2$ peak and ^{13}C peak 15, which is also correlated with the H8 peak, indicating that ^{13}C peak 15 is assignable to C7.

(9) ^{13}C peak 14s is not due to a carbonyl carbon; hence this peak is assigned to C6a, and this assignment is consistent with the correlation with proton peaks (H5"/H6' and H6").

(10) ^{13}C peak 14s is in relatively high field among singlet carbon nuclei. From chemical shift considerations, the only possible assignment is to C10a.

Now, remaining is the assignment of the signals for ^{13}C peaks 18-21, two β-carbons (peaks 18s and 19s) of the β-hydroxy-α,β-unsaturated ketone moiety and two carbonyl carbons (peaks 20s and 21s),

(11) The correlation between the H4 peak and ^{13}C peak 19 clearly indicates that peak 19 should be assigned to C3 and hence peak 18 to C12.

(12) The assignment of the two carbonyl peaks 20 and 21 was difficult because of overlapping of the H4a/H5a peaks. HMBC does not work in such a case, and thus we felt a deuterium isotope shift might be useful. In a mixed solvent of CD_3OD/DMSO-d_6, OH and NH_2 protons are exchanged slowly, and the chemical shifts of carbon nuclei nearby will be moved, in most cases, to high field.[6] There is an exchangeable proton three bonds apart from C1, that is, 12a-O<u>H</u>. From C11, all exchangeable protons (10-O<u>H</u> and 12-O<u>H</u>) are four bonds apart, and no isotope shift is expected. Indeed, peak 21 showed a small, but distinct, high field shift of *ca.* 0.2 ppm, while peak 20 did not show any such shift. Based on these observations, we assigned peaks 20 and 21 to C11 and C1, respectively, to complete the assignment of twenty-one ^{13}C peaks of **1**.

Our assignment together with that by Mazzola *et al*.[3] is given in Table 4.

Table 4. ^{13}C NMR Spectral Data for **1** in DMSO-d_6

peak no	chemical shift (δ) (this study)	assignment (this study)	chemical shift (δ) (ref.3.)	Assignment (ref. 3)
1	30.2t	C6	29.6t	C5
2	32.0d	C5a	31.6d	C5a
3	35.0t	C5	33.8t	C6
4	35.2d	C4a	35.1d	C4a
5	42.4q	4-N(CH_3)$_2$	41.2q	7-N(CH_3)$_2$
6	45.0q	7-N(CH_3)$_2$	44.4q	4-N(CH_3)$_2$

7	69.0d	C4	68.0d	C4
8	74.1s	C12a	73.9s	C12a
9	96.0s	C2	95.6s	C2
10	109.2s	C11a	108.3s	C11a
11	116.2s	C10a	115.6d	C9
12	116.5d	C8	116.0s	C10a
13	129.2d	C9	128.5d	C8
14	137.2s	C6a	136.6s	C7
15	142.1s	C7	142.2s	C6a
16	158.0s	C10	157.4s	C10
17	172.0s	2-$CONH_2$	171.9s	2-$CONH_2$
18	174.1s	C12	174.3s	C12
19	188.0s	C3	187.5s	C3
20	194.0s	C11	193.0s	C1
21	194.1s	C1	193.7s	C11

There are a few disagreements between the two assignments. We believe our assignment, based as it is on newer techniques, will be more dependable and we propose that the assignment of at least the following pairs, 4-$N(CH_3)_2$/7-$N(CH_3)_2$, C5/C6, C6a/C7 and C8/C9, should be reversed.

We are continuing our efforts to re-examine the NMR spectra, and modification with germanium moieties, of a variety of antibiotics.

Experimental Section

Minomycin hydrochloride (**1**), Minocin, $C_{23}H_{27}N_3O_7.2H_2O.HCl$, was obtained as a gift from Nippon Kayaku Co.

General Procedures. ^1H and ^{13}C NMR spectra of **1** were determined with a JEOL ECP-500 spectrometer in a 5 mm o.d. tube containing CD_3OD or DMSO-d_6 solution operating at 500 MHz and 125 MHz, respectively, using tetramethylsilane (TMS) as an internal standard. 0.050g of **1** was dissolved in 0.5 ml of CD_3OD or 0.014 g of **1** in 0.5 ml of DMSO-d_6. All the measurements were carried out in the manner described in the literature (HMQC[4], HMBC[5]) with the aid of the installed software.
J values are recorded in Hz.

Acknowledgements

This work was partly supported by The High-Tech Research Center Project from The Ministry of Education, Culture, Sports, Science and Technology.

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