Complete Assignment of 1H and 13C NMR Spectra of Tilmicosin Phosphate

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Abstract

The ^{1}H and ^{13}C NMR spectroscopic data of tilmicosin phosphate has been fully assigned by combination of one- and two-dimensional experiments (DEPT, HMBC, HMQC, ^{1}H-^{1}H COSY). The site of combination with phosphoric acid was assigned by comparing the ^{13}C NMR chemical shifts between tilmicosin and its phosphate. [Life Science Journal. 2010; 7(1): 107 – 110] (ISSN: 1097 – 8135).

Keywords: ^{1}H NMR; ^{13}C NMR; chemical shift; tilmicosin phosphate

1. Introduction

Tilmicosin, a semi-synthetic macrolide antibiotic derived from the macrolide antibiotic tylosin and 20-dihydro-20-deoxy-20-(*cis*-3, 5-dimethylpiperidin-1-yl) -desmycosin, is effective in the treatment of respiratory diseases in cattle against a broad range of bacteria. The free form is moderately soluble in aqueous solutions, while the chloride and phosphate salts are highly soluble[1]. Synthesis of tilmicosin was described by Debono et al[2,3].

The ^{1}H and ^{13}C NMR data of tilmicosin and its derivatives were discussed previously[4,5,6,7]. However, there is no literature precedent investigating the NMR data of tilmicosin phosphate.

The present study focused on the complete ^{1}H and ^{13}C NMR assignments of tilmicosin phosphate (Figure 1), and compared the ^{13}C NMR chemical shifts between tilmicosin and its phosphate, to assign the position which combined with phosphoric acid.

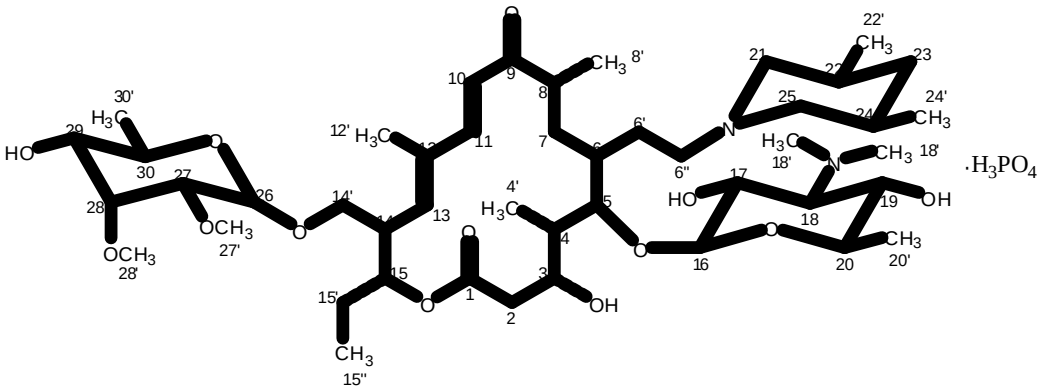

Figure 1. The structure of tilmicosin phosphate

2. Materials and Methods

Compound (Tilmicosin Phosphate) was provided by Xinxiang Ruite Fine Chemical Co. Ltd., Henan, China.

All NMR experiments were performed at 298K on a solution of the compound (40 mg) dissolved in 0.6 mL of D_2O on a Bruker Avance 300MHz instrument. ^{1}H NMR, ^{13}C NMR, quantitative ^{13}C NMR, DEPT-90, DEPT-135 NMR and ^{31}P NMR measurements were recorded with a 5 mm QNP probe. 2D NMR experiments were performed with a broadband inverse (BBI) probe equipped with a Z-gradient coil.

^{1}H NMR spectra were recorded at a frequency of 300.13 MHz with a spectral width of 6 kHz and a 7.20 μs (90°) pulse. The acquisition time was 2.7s and relaxation delay 2s; 16 scans with 32K data points were used. ^{13}C NMR, DEPT-90 and DEPT-135 spectra were recorded at a frequency of 75.47 MHz with a spectral width of 22.7 kHz and a 7.30 μs (90°) pulse. The acquisition time was 0.72s and relaxation delay 2s; 9k scans with 32K data points were used. The quantitative ^{13}C NMR spectrum was obtained using the zgig pulse program and with relaxation delay 20s, other parameters were as above. The HMQC spectra were obtained using the hmqcgpqf pulse program. The spectra resulted from a 256 x 1024 data matrix with 8 scans per t_1 increment. Spectral widths of 2480Hz in f_2 and 16650 Hz in f_1 were used. The acquisition time was 0.21s, the d2 delay was 3.45 ms (corresponding to an average $^{1}J_{(C,H)}$ of 145Hz) and the relaxation time was 2s. The HMBC spectra were obtained using the hmbcgplpndqf pulse program. The spectra resulted from a 256 x 1024 data matrix with 8 scans per t1 increment. Spectral widths of 2480Hz in f_2

and 16650 Hz in f_1 were used. The acquisition time was 0.21s, the d2 and d6 delays were set to 3.45 ms ($1/2J_{C,H}$) and 71.43 ms (corresponding to an average $1/^{n}J_{C,H}$ of 7 Hz) and the relaxation time was 2s. The ^{1}H-^{1}H COSY spectra were obtained using the cosydfph pulse program. The spectra result from a 256 x 1024 matrix with 8 scan per t_1 increment. Spectral widths of 2856 Hz in both f_1 and f_2 dimensions were used. The acquisition time was 0.20s and the relaxation time was 2s. ^{31}P NMR spectra were recorded at a phosphorus frequency of 121.44 MHz with a spectral width of 6 kHz and a 7.20 μs (90°) pulse.

Chemical shifts are given on the δ scale and are referred to 1,4-dioxane (external standard, sealed in a 1mm capillary) at δ = 3.70 ppm for ^{1}H, δ = 67.8 ppm for ^{13}C[10], and 85% H_3PO_4 (external standard, sealed in a 1mm capillary) at δ = 0.0 ppm for ^{31}P. Coupling constants *J* are reported in Hz. The following abbreviations were used: s, d and m, for singlet, doublet and multiplet, respectively.

3. Result and Discussion

The molecular formula of tilmicosin phosphate was deduced *as* $C_{46}H_{81}N_2O_{13}$ through HRMS, which showed an [M+1] peak at m/z 869.5732 (calcd for $C_{46}H_{81}N_2O_{13}$, 868.5660).

The 3,5-dimethyl-piperidino moiety was comprised of *cis*-isomer (≈85%) and *trans*-isomer (≈15%), the latter of which was excluded based on quantitative ^{13}C NMR spectrum. The quantitative ^{13}C NMR and DEPT spectra showed 44 signals (including 46 carbon atoms) comprising of 12 methyl, 9 methylene, 22 methine and 3 quaternary carbons, which confirmed the molecular formula.

The down field signal at δ 210.22 and 175.15 could be assigned to the ketone (C-9) and lactone (C-1) carbons. The presence of two olefinic bonds could be interred by signals at δ 150.36, 144.79, 136.73 and 119.29, respectively. Because the signal at δ 136.73 was a quaternary carbon, it could be assigned to C-12. The signals at δ 102.88 and 101.26 belong to the anomeric carbon atom. This indicated that there were two sugar molecules attached to tilimicosin. [8,9]

The ^{1}H NMR spectrum showed presence of the double bonds at δ 6.96 (1H, d, *J*=15.37Hz), 6.30 (1H, d, *J*=15.37Hz) and 5.61 (1H, d, *J*=10.25Hz), and the signal at δ 5.61 could be assigned to H-13. Two methoxy and two N-methyl protons were observed at δ 3.20 (3H, s), 3.10 (3H, s) and 2.63 (6H, s). Two signals at δ 4.25 (^{1}H, d, *J*=8.05Hz) and 4.17 (1H, d, *J*=7.32Hz) were assigned to the hydrogen atoms attached to the anomeric carbons by their chemical shifts and HMQC spectrum. [9]

The sginal at δ 2.81 (^{1}H, m) showed ^{1}H-^{1}H COSY correlations with H-13 was assigned to H-14. H-14 also showed ^{1}H-^{1}H COSY correlations with H-15 at δ 4.52. The proton at δ 4.25 showing ^{3}J correlations with two methane and one methylene was assigned to H-26. The other proton attached to anomeric carbon at δ 4.17 was assigned to H-16 and authenticated by HMBC. The coupling constants of H-16 and H-26 were 7.32Hz and 8.05Hz respectively, indicating β configuration to H-16 and H-26[9]. Other protons and carbons could be assigned by ^{1}H-^{1}H COSY, HMQC and HMBC.

H-4, H-6 and H-6' could not be identified in the ^{1}H NMR spectrum, because of the complexity of the coupling correlations and the large number of protons in the high field of the spectrum. Their chemical shifts were assigned by HMQC.

Table 1. ^{1}H and ^{13}C chemical shift and ^{1}H-^{1}H COSY, HMBC correlations of tilmicosin phosphate

position	δ (^{1}H), multiplicities and *J* (Hz)	δ (^{13}C)	^{1}H-^{1}H COSY	HMBC (H→C)
1	/	175.15	/	/
2	2.28 (m)	40.15	H-2, H-3	C-1, 3, 4, 4'
	1.85 (d, 17.93)		H-2	
3	3.34 (m)	67.77	H-2	C-1
4	1.48 (m)	41.27	/	/
4'	0.65 (m)	9.12	/	C-3, 4, 5
5	3.23 (m)	80.14	/	C-3, 4, 6, 6', 7
6	1.13 (m)	36.52	/	/
6'	1.66 (m)	23.74	/	/
	1.46 (m)			
6''	3.00 (m)	59.12	/	C-8
7	1.53 (m)	32.90	H-7, H-8	C-5, 8'
	1.19 (m)		H-7	
8	2.72 (m)	45.89	H-7, H-8'	C-8', 9
8'	0.90 (m)	17.28	H-8	C-7, 9
9	/	210.22	/	/
10	6.30 (d, 15.37)	119.29	H-11	C-9, 12
11	6.96 (d, 15.37)	150.36	H-10	C-9, 10, 12, 12', 13
12	/	136.73	/	/
12'	1.51 (s)	12.92	H-13	C-11, 12, 13
13	5.61 (d, 10.25)	144.79	H-12', H-14	C-11, 12', 14, 14', 15
14	2.81 (m)	45.22	H-13, H-15	C-14', 15
14'	3.64 (m)	70.56	H-14'	C-13, 14, 15, 26

	3.39 (m)			
15	4.52 (m)	76.58	H-14, H-15’	C-1, 15’’
15’	1.50, 1.28 (m)	25.19	H-15, H-15’’	C-15, 15’’
15’’	0.56 (m)	9.50	H-15’	C-15, 15’
16	4.17 (d, 7.32)	102.88	H-17	C-5, 20
17	3.37 (m)	69.01	H-16, H-18	C-16
18	2.97 (m)	70.87	H-17, H-19	C-17, 18’, 19
18’	2.63 (s)	42.03	/	C-18, 18’
19	3.18 (m)	69.49	H-18	C-20, 20’
20	3.17 (m)	73.01	H-20’	C-16, 19, 20’
20’	0.97 (m)	17.48	H-20	C-19, 20
21	2.98 (m)	50.00	H-21	C-22, 22’, 23
	2.15 (m)		H-21, H-22	
22	1.56 (m)	29.64	H-21, H-22’	C-24
22’	0.60 (m)	18.32	H-22	C-21, 22, 23
23	1.55 (m)	39.19	H-23	C-21, 22, 24
	0.51 (m)			
24	1.54 (m)	29.03	H-24’, H-25	/
24’	0.60 (m)	18.55	H-24	C-23, 24, 25
25	3.29 (m)	58.02	H-24, H-25	C-21, 23, 24, 24’
	2.10 (m)		H-25	
26	4.25 (d, 8.05)	101.26	H-27	C-14’, 28, 30
27	2.82 (m)	80.83	H-26, H-28	C-26, 27’
27’	3.10 (s)	58.69	/	C-27
28	3.60 (m)	79.61	H-27, H-29	C-26, 27, 28’, 29, 30
28’	3.20 (s)	62.25	/	C-28
29	3.01 (m)	73.23	H-28, H-30	C-28, 30, 30’
30	3.38 (m)	70.45	H-29, H-30’	C-26, 29, 30’
30’	0.87 (m)	17.48	H-30	C-29, 30

The signal of ^{31}P NMR at δ 2.077 showed the existence of phosphate.
The difference between tilmicosin[6,7] and its phosphate was shown in Table 2.

Table 2. The difference of ^{13}C NMR data between tilmicosin and its phosphate

position	δ (tilmicosin phosphate)	δ (tilmicosin[6], Acetone-d_6)	δ (tilmicosin[7], $CDCl_3$)
1	175.15	173.01	172.06
2	40.15	40.25	39.44
3	67.77	67.35	66.29
4	41.27	42.77	42.14
4’	9.12	9.30	9.195
5	80.14	80.03	79.20
6	36.52	32.86	31.55
6’	23.74	25.21	24.06
6’’	59.12	55.38	54.34
7	32.90	34.53	33.97
8	45.89	45.89	45.17
8’	17.28	18.15	17.87
9	210.22	203.62	203.92
10	119.29	119.81	118.12
11	150.36	147.95	147.76
12	136.73	135.31	134.39
12’	12.92	13.16	12.92
13	144.79	143.07	143.16
14	45.22	45.77	45.17
14’	70.56	69.41	69.30
15	76.58	74.79	73.76
15’	25.19	25.65	25.40
15’’	9.50	9.95	9.52
16	102.88	105.58	104.08
17	69.01	71.35	71.16
18	70.87	71.73	70.08
18’	42.03	42.03	41.69

19	69.49	71.91	70.85
20	73.01	73.94	73.16
20’	17.48	18.21	17.73
21	50.00	59.77	58.72
22	29.64	30.86	29.94
22’	18.32	19.88	19.48
23	39.19	43.47	42.35
24	29.03	30.65	29.80
24’	18.55	19.88	19.76
25	58.02	64.32	63.42
26	101.26	102.05	101.12
27	80.83	82.76	81.88
27’	58.69	59.41	59.72
28	79.61	81.41	79.82
28’	62.25	61.74	61.71
29	73.23	74.00	72.63
30	70.45	70.32	70.58
30’	17.48	17.92	17.73

4. Conclusion

The difference of chemical shifts of C-6’’, C-21 and C-25 between tilmicosin and its phosphate was larger than that of C-18 and C-18’, which indicated that the phosphoric acid was combined with the nitrogen atom of 3,5-dimethyl-piperdino group.

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