[Chem. Pharm. Bull. 36(10)4157—4161(1988)]

^{1}H- and ^{13}C-Nuclear Magnetic Resonance Spectra of Rifampicin and 3-[(Dimethylhydrazono)methyl]rifamycin SV

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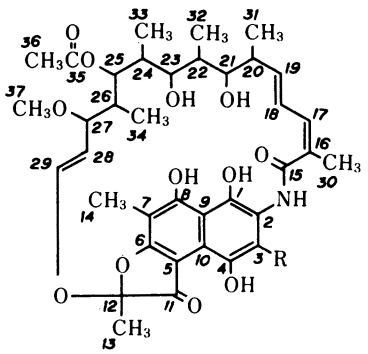
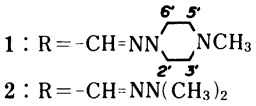
(Received March 22, 1988)

Assignments of labile protons (NH, OH-1, OH-4 and OH-8) and naphthalene carbon atoms in the ^{1}H- and ^{13}C-nuclear magnetic resonance spectra of rifampicin (**1**) and 3-[(dimethylhydrazono)-methyl]rifamycin SV (**2**) in $CDCl_3$ were made based on long-range selective decoupling experiments. The published assignments of the labile protons and two naphthalene carbon atoms (C-5 and C-9) of **1** should be revised.

Keywords——rifamycin; ^{1}H-NMR; ^{13}C-NMR; rifampicin

Rifamycins are important natural products, and signal assignments in the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra of naphthoquinone-type rifamycins have been discussed in detail.[1] Assignments of signals in the ^{13}C-NMR spectra for 1,4-naphthalenediol-type rifamycins including rifampicin[2] (**1**), a most valuable drug for the treatment of tuberculosis, have also been published,[3] but the basis of the assignments was not described. Recently, we reinvestigated the structure of the deep-blue compound, which was formed in the reaction of rifamycin S with 1,3,5-tri-*tert*-butylhexahydro-1,3,5-triazine,[4] and in connection with this work, it became necessary to confirm the published ^{13}C-NMR assignments of the naphthalene carbon atoms (C-1—C-10) of 1,4-naphthalenediol-type rifamycins. This paper describes the results of long-range selective proton decoupling (LSPD) experiments on **1** and 3-[(dimethylhydrazono)methyl]rifamycin SV (**2**), and points out that the published assignments[3b,c] of C-5 and C-9 of 1,4-naphthalenediol-type rifamycins should be interchanged. Signal assignments of labile protons (NH, OH-1, OH-4 and OH-8) in the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra of **1** and **2** are also described.





1 : R = -CH=NN(6' 5' 2' 3' NCH$_3$)

2 : R = -CH=NN(CH$_3$)$_2$

Chart 1

Results and Discussion

Chemical shifts and assignments in the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** in $CDCl_3$ are summarized in Tables I and II. Assignments of protons and carbon atoms of **1** and **2**, except for four labile protons in the chromophore (NH, OH-1, OH-4 and OH-8) and all quaternary carbon atoms, were made based on H–H and C–H correlation spectroscopy, and we confirmed the published assignments[3a,b,5a] of these protons and carbon atoms of **1**. In order to assign labile protons in the chromophore and all quaternary carbon atoms, proton non-decoupling spectra and LSPD spectra were recorded. The data are summarized in Tables

TABLE I. ^{1}H-NMR Chemical Shifts of Rifampicin (**1**) and 3-[(Dimethylhydrazono)methyl]rifamycin SV (**2**) in $CDCl_3$ (Concn. *ca.* 10^{-1} M) at 300 MHz

Compound	NH	OH-1	OH-4	OH-8	H-13	H-14	H-17	H-18	H-19	H-20	H-21
1	δ 13.19	12.8—13.8	11.98	12.8—13.8	1.80	2.21	6.39	6.57	5.93	2.37	3.77
2	δ 13.18	13.72	11.77	13.13	1.83	2.14	6.38	6.46	5.91	2.34	3.71

Compound	OH-21	H-22	H-23	OH-23	H-24	H-25	H-26	H-27	H-28	H-29	H-30
1	3.50	1.70	3.00	3.71	1.52	4.95	1.33	3.47	5.10	6.20	2.08
2	3.45	1.67	3.00	3.67	1.47	4.91	1.28	3.42	5.05	6.20	2.11

Compound	H-31	H-32	H-33	H-34	H-36	H-37	CH=N	H-2′,6′	H-3′,5′	NCH_3	$N(CH_3)_2$
1	0.88	1.01	0.59	−0.31	2.05	3.04	8.25	3.13	2.56	2.33	—
2	0.80	0.98	0.57	−0.39	2.03	3.01	7.71	—	—	—	2.90

TABLE II. ^{13}C-NMR Chemical Shifts of Rifampicin (**1**) and 3-[(Dimethylhydrazono)methyl]rifamycin SV (**2**) in $CDCl_3$ (Concn. *ca.* 10^{-1} M) at 75.47 MHz

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11
1	138.56	120.43	110.88	147.92	104.53	174.47	106.05	169.19	112.82	117.90	195.42
2	138.17	119.77	111.30	146.63	104.39	173.92	104.99	168.76	111.66	117.46	194.73

Compound	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20	C-21	C-22
1	108.74	21.40	7.50	169.67	129.37	135.05	123.21	142.64	38.54	70.62	33.36
2	108.65	21.42	7.30	169.47	129.47	134.34	122.99	142.05	38.28	70.37	33.10

Compound	C-23	C-24	C-25	C-26	C-27	C-28	C-29	C-30	C-31	C-32	C-33
1	76.89	37.49	74.41	39.48	76.74	118.64	142.62	20.66	17.80	10.84	8.42
2	76.57	37.28	74.18	39.38	76.57	118.59	142.63	20.54	16.52	10.52	8.24

Compound	C-34	C-35	C-36	C-37	CH=N	C-2′,6′	C-3′,5′	NCH_3	$N(CH_3)_2$
1	8.85	172.00	20.67	57.04	134.41	50.25	53.93	45.87	—
2	8.65	171.69	20.46	56.71	129.80	—	—	—	41.71

TABLE III. ^{13}C-NMR Chemical Shifts and Multiplicities of Quaternary Carbon Atoms in a Proton Non-decoupling Spectrum of Rifampicin (**1**) in $CDCl_3$ and Change of Multiplicities by LSPD

Chemical shifts	Multiplicities (*J*, Hz)	Assignments	Change of multiplicities (*J*, Hz) by LSPD			
			δ2.21 (H-14)	δ8.25 (CH=N)	δ11.98 (OH-4)	δ13.19 (NH)
195.42	q (2)	C-11	—	—	—	—
174.47	q (4)	C-6	s	—	—	—
172.00	dq (4, 7)	C-35	—	—	—	—
169.67	dqd (1, 4, 12)	C-15	—	—	—	qd (4, 12)
169.19	q (4)	C-8	s	—	—	—
147.92	t (2)	C-4	—	d (2)	d (2)	—
138.56	dd (1, 5)	C-1	—	d (5)	—	d (1)
129.37	dq (1, 7)	C-16	—	—	—	—
120.43	d (6)	C-2	—	s	—	—
117.90	d (1)	C-10	—	—	s	—
112.82	s	C-9	*a*)	—	—	—
110.88	dt (3, 6)	C-3	—	dd (3, 6)	dd (3, 6)	t (6)
108.74	quintet (4.5)	C-12	—	—	—	—
106.05	q (6)	C-7	s	—	—	—
104.53	s	C-5	—	—	—	—

a) The peak height was clearly enhanced.

TABLE IV. ^{13}C-NMR Chemical Shifts and Multiplicities of Quaternary Carbon Atoms in a Proton Non-decoupling Spectrum of 3-[(Dimethylhydrazono)methyl]rifamycin SV (**2**) in $CDCl_3$ and Change of Multiplicities by LSPD

Chemical shifts	Multiplicities (*J*, Hz)	Assign-ments	Change of multiplicities (*J*, Hz) by LSPD					
			δ2.14 (H-14)	δ7.71 (CH=N)	δ11.77 (OH-4)	δ13.13 (OH-8)	δ13.18 (NH)	δ13.72 (OH-1)
194.73	q (3)	C-11	—	—	—	—	—	—
173.92	dq (2, 4)	C-6	d (2)	—	—	q (4)	—	—
171.69	dq (4, 7)	C-35	—	—	—	—	—	—
169.47	qd (4, 11)	C-15	—	—	—	—	—	—
168.76	quintet (4)	C-8	d (4)	—	—	q (4)	—	—
146.63	t (2)	C-4	—	d (2)	d (2)	—	—	—
138.17	ddd (1, 3.5, 4.5)	C-1	—	dd (3.5, 4.5)	—	—	dd (1, 3.5)	dd (1, 4.5)
129.47	q (7.5)	C-16	—	—	—	—	—	—
119.77	d (6)	C-2	—	s	—	—	—	—
117.46	d (1)	C-10	—	—	s	—	—	—
111.66	t (4)	C-9	*a*)	—	—	d (4)	—	d (4)
111.30	dt (3.5, 6)	C-3	—	dd (3.5, 6)	dd (3.5, 6)	—	t (6)	—
108.65	quintet (4)	C-12	—	—	—	—	—	—
104.99	quintet (6)	C-7	d (6)	—	—	q (6)	—	—
104.39	s	C-5	—	—	—	—	—	—

a) The peak height was clearly enhanced.

III and IV. Assignments were made as follows.

Assignments of C-11, C-12, C-15, C-16 and C-35

Assignments of these five quaternary carbon atoms were made with reference to the published assignments[3a,b] of **1**, and were confirmed by the multiplicities of the corresponding signals (Tables III and IV).

Assignments of C-1, C-2, C-3, C-4, C-10 and Labile Protons in the Chromophore

Assignments for 1——LSPD of the hydrazonomethyl proton (CH=N) affected four signals at 147.92, 138.56, 120.43 and 110.88, and hence these signals must be attributed to C-1, C-2, C-3, C-4 or C-10. The two lower signals at 147.92 and 138.56 are attributed to C-1 and C-4, because these carbons each bear an oxygen atom. The ^{1}H-NMR spectrum of **1** showed two sharp singlets at δ 11.98 and 13.19 (each corresponding to one proton) and one very broad signal in the range of δ 12.8—13.8 (corresponding to two protons), due to four labile protons in the chromophore (NH, OH-1, OH-4 and OH-8). The singlet at δ 13.19 is assigned to NH, because LSPD of the δ 13.19 proton affected the signal at 169.67 (C-15). The LSPD of the same proton also affected the signal at 138.56, and this result allowed us to assign the signal at 138.56 to C-1. The signal at 147.92 is therefore assigned to C-4. LSPD of the δ 11.98 proton affected the signal at 147.92 (C-4), and hence the singlet at δ 11.98 is assigned to OH-4. The remaining broad signal in the range of δ 12.8—13.8 is therefore assigned to OH-1 and OH-8. These assignments of labile protons in the chromophore are in disagreement with the assignments in the literature.[3a,b,5] The published assignments of these protons should be revised as above. The signal at 110.88 was affected by LSPD of the hydrazonomethyl proton (CH=N), the amidic proton (NH), and the proton of the 4-hydroxyl group, and is assigned to C-3. The remaining signal at 120.43 is assigned to C-2, because the signal at 117.90 is assigned to C-10 which was affected by LSPD of the proton of the 4-hydroxyl group. These assignments of C-1, C-2, C-3, C-4 and C-10 are in agreement with the published assignments.[3b]

Assignments for 2——Assignments of C-1, C-2, C-3, C-4 and C-10 of **2** were made with reference to the assignments described above for **1**. Differing from **1**, the ^{1}H-NMR spectrum of **2** showed four sharp singlets due to four labile protons in the chromophore (NH, OH-1, OH-4 and OH-8), and assignments of these labile protons in the chromophore were made by LSPD experiments. The results are shown in Table IV.

Assignments of C-5, C-6, C-7, C-8 and C-9

Assignments for 1——Assignments of C-5, C-6, C-7, C-8 and C-9 were made with reference to assignments described below for **2**, and the assignments of C-5 and C-9 were found to be in disagreement with the published assignments.[3b] The published assignments of C-5 and C-9 should be interchanged as shown in Tables II and III.

Assignments for 2——Among fifteen signals due to quaternary carbon atoms, the five signals at 173.92, 168.76, 111.66, 104.99 and 104.39 must be attributed to C-5, C-6, C-7, C-8, and C-9. The two signals at 173.92 and 168.76 are attributed to two carbons, C-6 and C-8, bearing an oxygen atom. The coupling constants between these carbons and the proton of the 8-hydroxyl group allowed us to assign the signal at 173.92 to C-6 and the signal at 168.76 to C-8, because the long-range coupling constant ^2J is generally larger than ^4J.[6] The result of LSPD of H-14 allowed us to assign the signal at 104.99 to C-7. The signal at 111.66 was affected by LSPD of the protons of the 1-hydroxyl and the 8-hydroxyl groups, and hence this signal is assigned to C-9. The remaining signal at 104.39 must be attributed to C-5. These assignments mean that the published assignments[3b,c] of C-5 and C-9 of 1,4-naphthalenediol-type rifamycins should be interchanged.

Experimental

Compounds **1** and **2** were prepared according to the literature.[7,8] The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-300 spectrometer in $CDCl_3$ solution, and tetramethylsilane (TMS) was used as an internal reference. Typical experimental conditions are as follows. For ^{1}H-NMR spectra: pulse width 4 μs (15 μs for a 90° pulse); sweep width 6000 Hz at 300 MHz; relaxation delay 1 s; acquisition time 2.72 s; 32 k memory. For ^{13}C-NMR spectra: pulse width 3.6 μs (7 μs for a 90° pulse); sweep width 18500 Hz at 75.47 Hz; relaxation delay 1 s; acquisition time 1.769 s; 64 k memory. The resultes are summarized in Tables I—IV.

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