CARBON-13 SPIN-LATTICE RELAXATION TIMES AND CONFORMATION OF ROSARAMICIN

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(Received for publication January 28, 1981)

During the past year we have been interested in the determination of carbon-13 spin-lattice relaxation times of biologically important macrolides such as megalomicins[1]. Relaxation times (T_1, second) data are useful in the understanding of conformational and/or isotropic motion of the molecule in solution. We report here the conformation and relaxation behavior of rosaramicin ($C_{31}H_{51}NO_9$, *m/z* 581.3569) (**1**), a potent macrolide antibiotic elaborated by *Micromonospora rosaria*.[2]

Conformation of Rosaramicin in Solution

Proton (100 MHz) NMR spectra of rosaramicin was studied in deuterium oxide, chloroform-*d*, acetone-d_6, and chloroform - benzene mixtures. In the latter system, all resonances, except for H_{20}, H_{11}, H_3, H_5, and the anomeric protons, gradually shifted upfield from $CDCl_3$ to C_6D_6 solutions indicating two sites of interactions along the enone and the aldehydic functions.

Proton NMR spectra in acetone-d_6 are shown in Fig. 1. Extensive decoupling as well as high resolution (300 and 600 MHz) studies resulted in complete stereochemical assignments of fragments A and B shown above. Analysis of the data, including dihydral angles (ϕ) calculated with the aid of KARPLUS equation[3], indicate that pairs of protons H_{10} and H_{11} ($J_{10,11}$=16.0 Hz), H_{13} and H_{14} ($J_{13,14}$=10.0 Hz) and H_{14} and H_{15} ($J_{14,15}$=8.0 Hz, ϕ=160°) are *trans* to each other. In addition, H_{15} is further coupled to two non-equivalent (J_{gem}=14.0 Hz) methylene protons H_{16A} ($J_{15,16A}$=9.0 Hz, ϕ=170°, *trans*) and H_{16B} ($J_{15,16B}$=3.0 Hz, ϕ=50°, *cis*). The methylene protons are further coupled to protons of 17-CH_3 group.

Fig. 1. Proton NMR spectrum (100 MHz) of rosaramicin in acetone-d_6 (A) and its 500 Hz expansion (B).

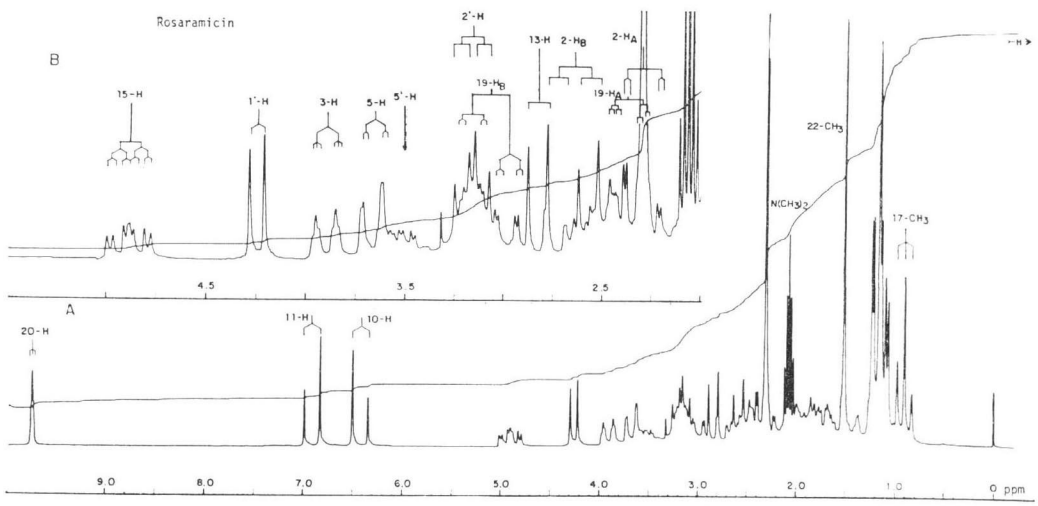


Table 1. ^{13}C Chemical shifts and spin-lattice relaxation times of rosaramicin and its salt.

Carbon	δ, ppm[a]	T_1 (second)[a]	T_1 (second)[b]
1	173.5	5.6[c]	—
2	39.7	0.23	0.10
3	68.0	0.44	0.17
4	45.1	0.39	0.15ϕ
5	81.3	0.40	0.16
6	31.4	0.29	0.12
7	31.8	0.17	0.08
8	37.9	0.39	0.17
9	200.9	11.6[c]	—
10	122.8	0.41	0.18
11	150.9	0.46	0.15
12	59.7	6.0[c]	—
13	66.8	0.41	0.25*
14	41.3	0.45	0.14
15	76.8	0.45	0.13
16	24.7	0.28	0.12
17	9.0	0.51*	0.78
18	9.1	0.51*	0.43
19	43.8	0.23	0.15ϕ
20	202.9	0.93[c]	0.81
21	17.4	0.78[c]	0.42
22	15.0	1.16[c]	0.53
23	14.5	0.61	0.36
1′	104.5	0.39	0.24
2′	70.4	0.45	0.22
3′	65.4	0.39	0.19
4′	28.4	0.22	0.08
5′	69.7	0.39	0.25*
6′	21.2	0.63[c]	0.49
7′, 8′	40.5	0.58	0.43

[a] Free base in $CDCl_3$.
[b] NaH_2PO_4 Salt in D_2O.
[c] Values from additional experiment.
* and ϕ values are interchangeable with the same type.

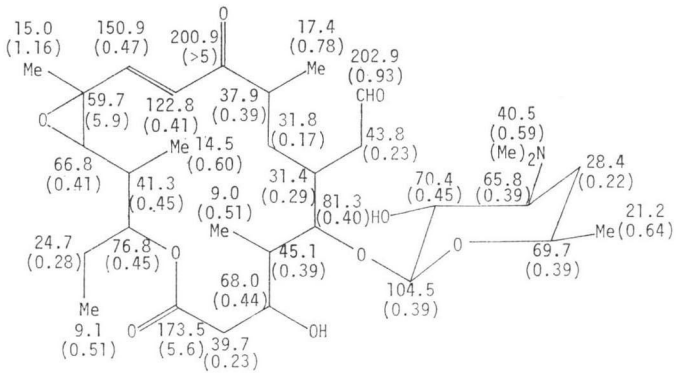
Non-equivalent (J_{gem}=17.5 Hz) methylene protons H_{2A} and H_{2B} are *cis* ($J_{2A,3}$=1.5 Hz, ϕ=65°) and *trans* ($J_{2B,3}$=10.0 Hz), respectively, to H_3 which is further *cis* ($J_{3,4}$=1.5 Hz, ϕ=65°) to H_4. Proton H_4 is *trans* ($J_{4,5}$=10.0 Hz) to H_5 which in turn is *cis* ($J_{5,6}$=1.5 Hz, ϕ=65°) to H_6. Proton H_6 is a multiplet of considerable complexity. On one hand, it is coupled to two non-equivalent (J_{gem}=17.5 Hz) methylene protons H_{19A} ($J_{19A,6}$=3.0 Hz, ϕ=50° *cis*) and H_{19B} ($J_{19B,6}$=10.0 Hz, *trans*) which are further identically coupled (J=1.5, 1.5 Hz) to the aldehydic proton H_{20}. On the other hand, H_6 also is *trans* ($J_{6,7A}$=12.5 Hz) and *cis* ($J_{6,7B}$=2.0 Hz, ϕ=60°) to 7-CH_2 non-equivalent (J_{gem}=13.0 Hz) protons H_{7A} and H_{7B} which are further coupled *cis* ($J_{7A,8}$=3.0 Hz, ϕ=50°) and *trans* ($J_{7B,8}$=10.0 Hz) to H_8. The desosamine ring possessed all *trans* stereochemistry[4]. The chemical shifts (δ, ppm) and coupling constants (Hz) of the desosamine protons were, CH_3=1.18 (d, J=6.5), $N(CH_3)_2$=2.30 s, H_{1a}=4.36 (d, $J_{1,2}$=7.0), H_{2a}=3.17 (dd, $J_{2,3}$=10.0), H_{3a}=2.55 (m, $J_{3,4a}$=12.5, $J_{3,4e}$=4.0), H_{4a}=1.20 (m, $J_{4a,4b}$=13.0, $J_{4a,5}$=10.0), H_{4e}=1.72 (m, $J_{4e,5}$=2.0) and H_{5a}=3.55 (m). Molecular models as well as recent X-ray crystallographic data of rosaramicin[5] and tylosin[6] support the NMR analysis.

T_1 Relaxation Data

The nuclear Overhauser enhancement (N.O.E.) value for **1** was 2.06 indicating relaxation mechanism to be completely dominated by ^{13}C-^1H dipolar interactions. Carbon-13 relaxation data of **1** in $CDCl_3$ and D_2O are presented in Table 1 and Fig. 2 ($CDCl_3$ only). Carbon-13 shift assignments of **1** were the subject of earlier reports[7] and, therefore, will not be discussed. The average NT_1 (N=number of directly attached protons) values in $CDCl_3$ for CH and CH_2 carbons of aglycone and sugar of 0.40±0.05 and 0.41±0.03 second, respectively, suggest that **1** undergoes isotropic motion in solution as a whole. Desosamine has the same degree of freedom as the aglycone[8]. In the case of sodium dihydrogen phosphate salt of **1** in D_2O, the NT_1 values of 0.17 and 0.21 second for aglycone and sugar, respectively, indicate significant reduction in the mobility of the macrolide. The effect of solute-solvent interactions (such as hydrogen-bonding) coupled with tighter aqueous solvation and greater viscosity of D_2O are responsible for the reduced molecular motion of the solute; the latter plays a major role[9].

Particularly, the T_1 values of **1** indicate that the 15-CH_2CH_3 and 6-CH_2CHO moieties exhibit considerable freedom of motion in solution, *e. g.*, T_1 of 0.93 second for CHO and 0.12 and 0.78 second (in D_2O) for CH_2 and CH_3 carbons, respectively, are larger than the calculated NT_1 values (T_1CHO>0.40 and T_1CH_3>0.51 second). Methyl groups experience some restriction to their motional freedom, except the C_{12}–CH_3 group whose high T_1 value (1.16 second) shows it

Fig. 2. Carbon-13 chemical shifts with T_1 (second) relaxation times in paranthesis of rosaramicin in $CDCl_3$.



Rosaramicin (**1**)

to behave as a freely rotating function like the β-axial 18 or 19 methyl groups in steroids ($T_1(CH_3)$/$T_1(CH)=3$) which relax *via* dipole-dipole interactions[10].

Experimental

Proton NMR spectra and decoupling experiments were carried out utilizing Varian Associates XL-100-15 and SC-300 spectrometers. 600 MHz spectra was obtained from Carnegie Mellon University, Pittsburgh, PA. Carbon-13 T_1 relaxation experiments were carried out at 20 MHz utilizing Varian FT-80A spectrometer[8].

The inversion-recovery pulse sequence, (180°–t_1–90°–T)n, was utilized to calculate T_1 data. For a typical experiment using 8k data point, the spectrometer settings were: sweepwidth=5000 Hz, acq. time=0.819 second, 90° pulse=22 μsec, 180° pulse=44 μsec and pulse delay=6 seconds. The values of t_1 (second) were 0.010, 0.020, 0.040, 0.080, 0.160, 0.320, 0.640, 1.281, 2.562, and 5.125. The samples were available as chromatographically separated materials.

Acknowledgements

The author wishes to thank Dr. R. BRAMBILLA for his technical assistance and Prof. A. A. BOTHNER-BY for the 600 MHz spectra. NIH is thanked for the support (Grant No. RR00292) of 600 MHz NMR facility at Carnegie Mellon University, Pittsburgh, PA, U.S.A.

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