Carbon-13 Spin–Lattice Relaxation Times of Megalomicins, Fourteen-membered Macrolide Antibiotics

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Spin–lattice relaxation times and their application for spectral analysis of megalomicins are discussed. T_1 Values of megalomicins and erythromycin are analysed in terms of molecular motions in solution.

SPIN–lattice relaxation times (T_1) and their use for spectral analysis of complex molecules such as erythromycins have been reported.[1-3] These data are invaluable not only in the assignment of carbon chemical shifts but also in the understanding of conformational mobility and/or isotropic motion of the molecule. In addition T_1 data provide an important method of differentiating the macrolide carbons from the saccharides.

We report here the T_1 relaxation behaviour of megalomicin A (1) and megalomicin C_2 (3'''-acetyl-4'''-propionylmegalomicin A) (2) and compare it with erythromycin A (4) *via* a common intermediate, (9S)-5-*O*-β-D-desosaminyl-9-dihydroerythronolide (3). These macrolides have a common aglycone which differ only in the number and structures of the attached sugars. Compound (3) was used to establish structural relationship between erythromycin and megalomicin in earlier studies.[4]

RESULTS AND DISCUSSION

The nuclear Overhauser enhancements (N.O.E.) for compounds (1)—(3) indicate that all protonated carbons in addition to some of the non-protonated carbons experienced full N.O.E. The average values were 1.9, 1.8, and 2.0, respectively. The relaxation mechanism is, therefore, completely dominated by dipole–dipole interactions with the attached protons.

The average NT_1 (N = number of attached protons) values of the respective fragments, obtained from methine and methylene carbons are given in the Table.

Average ^{13}C NT_1 data (in s) for 14-membered macrolide antibiotics

Carbon type (CH + CH_2)	Megalomicin A (1)	Megalomicin C_2 (2)	Intermediate (3)	Erythromycin A [a] (4)
Macrolide ring	0.17 ± 0.03	0.20 ± 0.03	0.29 ± 0.04	0.23 ± 0.03 (0.34)
Desosamine	0.24 ± 0.02	0.24 ± 0.04	0.36 ± 0.02	0.29 ± 0.02 (0.40)
Mycarose	0.19 ± 0.03	0.22 ± 0.03		
Cladinose				0.24 ± 0.02 (0.40)
Megosamine	0.17 ± 0.01	0.22 ± 0.02		

[a] The values in parentheses are from ref. 1.

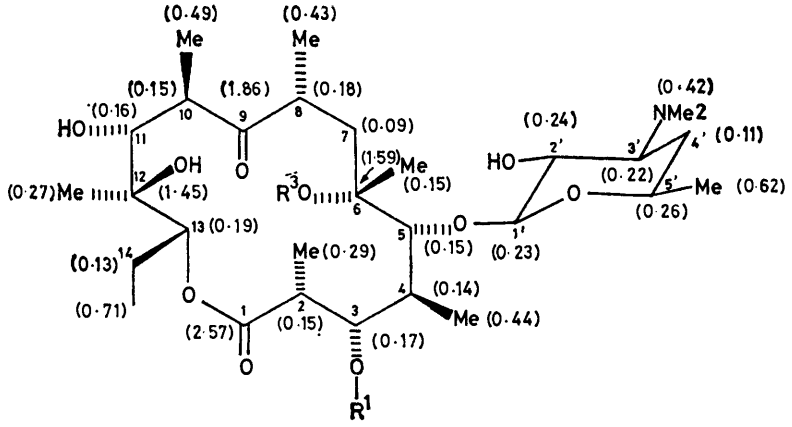
The fact that NT_1 values of the macrolide ring, mycarose and megosamine of (1) are essentially identical suggests that these fragments exist as a globular molecule which moves around isotropically in solution, whereas desosamine exhibits a motional freedom in solution. Even though the average NT_1 values for (1) and (2), except for megosamine, overlapped each other, a comparison of the NT_1 values of (1) and (2) suggests that esterification of the two OH groups of mycarose minimizes hydrogen bonding to the macrolide ring and/or to the solvent and imparts a greater degree of motional freedom for (2).

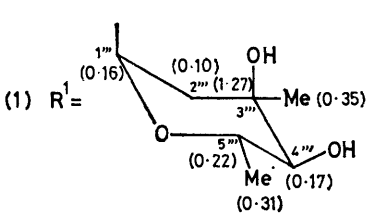
Compound (3) is derived from megalomicin A (1) and erythromycin A (4) and reflects the basic isotropic motional behaviour of the aglycone–desosamine molecule in solution. Further glycosidation at C-3 as in (4) or C-3 and C-6 as in (1) and (2) produced a marked shortening of the average NT_1 values consistent with additional restraints such as association imposed upon the antibiotic molecule in solution.

In all these antibiotics desosamine experiences an extra degree of freedom of motion and its role in the binding to the ribosomal surface has already been implicated.[3] These observations are in agreement with *X*-ray crystallographic data of 4''-*O*-(4-iodobenzoyl)-megalomicin A which shows that mycarose and megosamine are in closer proximity to the aglycone than desosamine.[5]

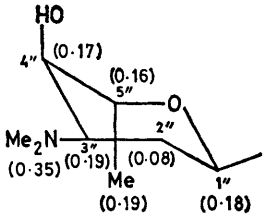
Specially, the T_1 values for megalomicin A (1) indicate that the methyl groups attached to quarternary carbons (6-, 12-, and 3'''-Me) as well as the methyl groups not exposed to the surface of the solvent (2-, 5''-, and 5'''-Me) have the smallest T_1 values. The methyl groups are expected to have T_1 values of *ca.* 0.51 s [3 T_1 (CH) = 0.51 s] and smaller experimental values suggest that their mobility is restricted. The methyl groups at C-6 and C-5'' with T_1 values of 0.14 and 0.19 s, respectively, are viewed as the most sterically hindered functions. Other methyl groups undergo normal internal rotation. On the other hand, the ethyl group at position 13 moves freely around both bonds even though $T_1(CH_2)$ was taken along with the aglycone for the calculations of average NT_1 values. T_1 Values of 0.13 and 0.71 s for the methylene and methyl carbons, respectively, are larger than the calculated ones.

In the case of erthyromycin A (4), our data indicate





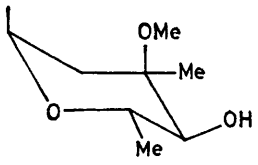
L- mycarose



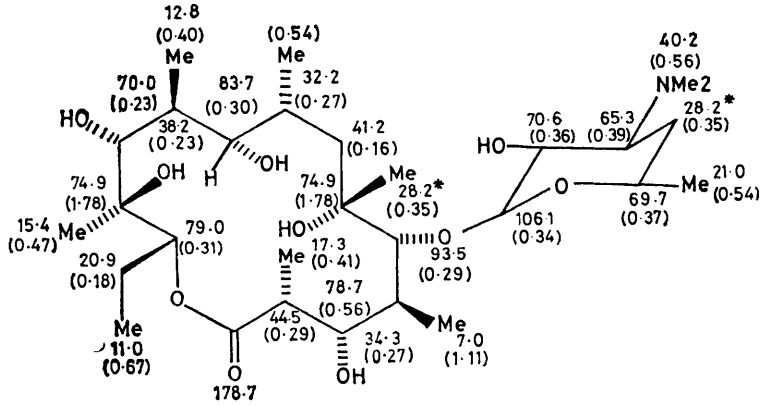
L- megosamine

(2) R^1= 3‴- acetyl - 4‴- propionyl - L- mycarose, R^3= L- megosamine

(4) R^1=



L-cladinose (3‴-OMe- mycarose), R^3= H



(3)

T_1 Values in parentheses

$T_1(CH_3)$ values of *ca.* 0.5 s which suggest conformational uniformity [$T_1(CH_3) > 2T_1(CH)$]. Only 12-Me (T_1 0.19) was present in an extremely hindered environment. Once again the 13-ethyl side-chain exhibited freedom of rotation [$T_1(CH_2)$ 0.19 s and $T_1(CH_3)$ 0.84 s]. The difference in the NT_1 values of (4) from the literature data are attributed to experimental conditions of concentration and temperature.*

We expected the conformation of the agylcone to change after the hydrolysis of two sugars in (3) and the T_1 values reflect that change. The methyl groups at positions 2, 6, 8, 10, and 12 experience some constraint. On the basis of T_1 relaxation determination of (3) the following chemical shifts (p.p.m.) are revised: C-4 (34.3), C-5 (93.5), C-7 (41.2), C-8 (32.2), C-10 (38.2), and 6-Me (28.2).†

EXPERIMENTAL

^{13}C N.m.r. spectra were recorded using a Varian FT-80A spectrometer operating at 20 MHz and at ambient temperature. The concentrations of the solutions were close to 30% w/v in deuteriochloroform. For a typical experiment using 8K data point, the spectrometer settings were: sweepwidth 5 000 Hz, acquisition time 0.819 s, 90° pulse 22 μs, 180° pulse 44 μs, and pulse delay 6 s. The values of t_1 were 0.010, 0.020, 0.040, 0.080, 0.160, 0.320, 0.640, 1.281, 2.562, and 5.125 s.

Nuclear Overhauser enhancement measurements were carried out utilizing two experiments, one with the decoupler on all the time and the other with the decoupler on only during the 90° pulse and the acquisition time.

The inversion–recovery pulse sequence, $(180°–t_1–90°–T)n$, was utilized to calculate the T_1 data. T_1 Calculations were carried out by utilizing a three-parameter non-linear least-squares fit for the best values of T_1, M_0, and K (ideally $K = -1$ for inversion-recovery experiment) to equation (1).

$$M_t = M_0(1 + 2ke^{-t/T_1}) \quad (2)$$

Varian FT-80A applications software programs TICALC and ANALYZER were used and the errors of the measured T_1 values were better than ±10%. The errors in the average NT_1 values are standard deviations. Differences between the average NT_1 values were also tested for 95% confidence limit using Duncan's range test. T_1 Values of some of the quaternary carbons were not measured.

The samples were available as chromatographically separated and analytically pure materials.[4,5]

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* It has not been possible to investigate this point further. However, we feel our data are consistent throughout.

† T_1 Values of C-4, C-8, and C-10 are more consistent with the T_1 values of the adjacent methine carbons and more accurately reflect the motional freedoms of the aglycone ring. Perhaps the T_1 and chemical shift values of 4-Me should be interchanged with 14-Me.

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