Carbon-13 Spin–Lattice Relaxation Times and Their Use for Spectral Analysis of 16-Membered Macrolide Antibiotics[1]

By ANDRAS NESZMELYI

(*The Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Budapest, Pusztaszeri ut, Hungary*)

SATOSHI OMURA

(*The Kitasato Institute and Kitasato University, Minato-ku, Tokyo* 108, *Japan*)

and GABOR LUKACS*

(*Institut de Chimie des Substances Naturelles du C.N.R.S.*, 91190 *Gif-sur-Yvette, France*)

Summary The carbohydrate carbon resonances of the macrolide antibiotics studied can be in most cases differentiated from the aglycone signals by carbon-13 spin–lattice relaxation time measurements.

UNAMBIGUOUS assignment of all the resonances in the proton-decoupled ^{13}C n.m.r. spectra of complex natural products often becomes a necessity for structural or conformational analysis, but in some cases this may be very difficult. Besides the well known conventional techniques[2] ^{13}C n.m.r. spin–lattice relaxation time measurements can be invaluable in this respect when internal or anisotropic motion contributes substantially to T_1 for some carbon atoms.[3]

A number of biologically important compounds are made up of a complex aglycone to which one or several sugar units are attached. An analysis of the ^{13}C n.m.r. spectra of these substances often requires spectral data for the appropriate methyl glycosides as models.[4] These compounds may not be easily available, and may require special syntheses. We show here that in some cases the carbohydrate carbon resonances can be differentiated from the aglycone signals by ^{13}C n.m.r. spin–lattice relaxation time measurements without spectral comparison between the natural product and its sugar components.

The complete set of measured T_1 values† for the macrolide antibiotic tylosin (**1**) is shown in the Figure while the Table indicates the average ^{13}C n.m.r. NT_1 data for the CH

† N.O.E. determinations indicate that relaxation of the protonated carbon atoms is overwhelmingly dominated by dipolar interactions with the attached protons.

and CH_2 type carbon atoms of the compounds examined. The average NT_1 values corresponding to the respective fragments given in the Table were calculated on the basis of our previously reported chemical shift assignments.[4] In tylosin (**1**) and chalcomycin (**4**) NT_1 values for the mycinose carbons are markedly longer than the NT_1 values calculated for the respective aglycones or other hexoses present in these

TABLE[a]

Average ^{13}C NT_1 data (in sec) for 16-membered macrolide antibiotics[b]

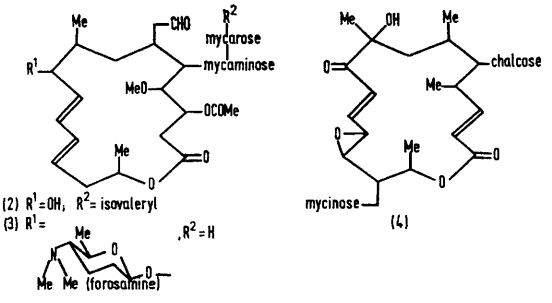
Carbon type (CH + CH_2)	Tylosin (**1**)	Leucomycin A_3 (**2**)	Spiramycin III (**3**)	Chalcomycin (**4**)
Macrolide ring ..	0·16	0·09	0·13	0·30
Mycaminose ..	0·17	0·09	0·15	—
Mycarose	0·20	0·11	0·19	—
Mycinose	0·37	—	—	0·45
Forosamine ..	—	—	0·23	—
Chalcose	—	—	—	0·34

[a] ^{13}C N.m.r. spectra were recorded for 0·1—0·5 M solutions in $CDCl_3$ on a Varian XL-100-15 F.T. spectrometer equipped with a Varian 630/1 computer. For atoms with short T_1 values (< 0·4 s) the inversion recovery technique was employed while longer relaxation times were measured by progressive saturation. Reproducibility of the measured T_1 values was ±5—10%. The T_1 values were computed in two steps. First, all individual nuclei were fitted by a two-parameter non-linear least-squares program to the following equation (R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, 1971, **54**, 3367).

$$y(t) = y_\infty\,[1 - \exp(-t/T_1)]/[1 - k\exp(-t/T_1)]$$

In the second step a multiparameter fitting was done to eliminate minor variations in amplification factors, *etc.* during the measurements. Thus all the nuclei were fitted simultaneously with the starting parameters determined in the first step. [b] The average hexose–macrolide NT_1 ratio seems to be concentration-dependent. Chalcomycin was examined in 0·4 M solution (mycinose/macrolide ring NT_1 = 1·5) and tylosin in 0·25 M solution (mycinose/macrolide ring NT_1 = 2·3).

molecules. Since mycinose is attached to the aglycones by means of an –O–CH_2– linkage it has faster internal reorientation than the other rings in analogy with the relaxation behaviour of the terminal galactose unit of stachyose.[3a] Furthermore, it is important to note that in chalcomycin (**4**) and spiramycin III (**3**), as a result of the difference in ring size, the chalcose and forosamine carbon atoms show higher NT_1 values than the macrolide carbon atoms. On the basis of our previous study[4] C-15 and C-5′ of the chalcose unit of (**4**) could not be distinguished. The measured T_1 values for these resonances (68·5 p.p.m.; 0·25, and 67·5 p.p.m.; 0·35 s) permit an unambiguous assignment to be made (Table). The lower-field shift must be attributed to C-15 and the higher field resonance to the chalcose carbon atom.‡



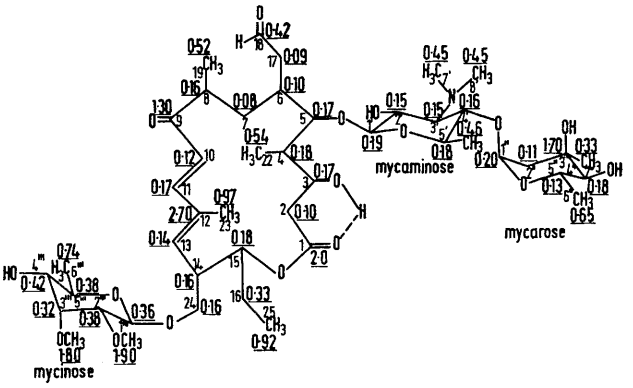


FIGURE. ^{13}C Spin–lattice relaxation times for tylosin (**1**) (in sec) (underlined numbers). Other numbers designate the carbon chain.

In the case of tylosin (**1**), leucomycin A_3 (**2**), and spiramycin III (**3**) a mycarosyl–mycaminose disaccharide unit is attached to the respective aglycones. The mycaminose carbon atoms cannot be recognized in these compounds since the corresponding T_1 values are shortened by the presence of a mycarose unit. In view of the terminal disposition of mycarose, the carbon atoms of this sugar unit show slightly longer T_1 values (Table) than the mycaminose carbon atoms of these molecules. However, these differences are only small and they suggest that care should be exercised in similar cases when T_1 is used in resonance assignments.

The resonance identification technique described in this investigation should be of great help in the ^{13}C n.m.r. spectral analysis of a large variety of antibiotics and other natural products.

(*Received, 5th September* 1975; *Com.* 1006.)

‡ Both these resonances represent CH type carbons. The chalcose carbon is designated by the prime symbol.

[1] For previous paper in the series: 'Résonance Magnétique Nucléaire du ^{13}C de Produits Naturels et Apparentés,' see S. Omura, A. Neszmelyi, M. Sangaré, and G. Lukacs, *Tetrahedron Letters*, 1975, 2939.
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