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A CARBON - 13 SPIN-LATTICE RELAXATION TIME STUDY OF 14-MEMBERED MACROLIDE ANTIBIOTICS [1].

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Three independent publications appeared last year dealing with the ^{13}C N.M.R. spectral analysis of 14 - membered macrolide antibiotics [2a,2b,2c]. In view of these highly complicated systems major differences are evident in the interpretation of the spectral data by the three groups. The differences, having conformational implications, are essentially related to the assignment of the C-methyl resonances and to the spectrum of lankamycin 1. The C-methyl carbons of these compounds are all biogenetically derived from C-3 of a propionate unit [3]. As a consequence, a biogenetically enriched antibiotic from a [3 - ^{13}C] propionate is not expected to yield information for the specific assignment of the C-methyl signals. Therefore, we tried another approach to the problem by using carbon - 13 spin-lattice relaxation times and in this letter we wish to present our results.

It is well known that the aglycone of the 14 - membered antibiotics is of a slightly modified diamond-lattice type with the ring substituents occupying axial and equatorial-like configurations [4a,4b]. ApSimon et al. [5a] and Wehrli [5b] have shown recently that the spin-lattice relaxation time of a methyl group depends on the number of 1,3-diaxial methyl group-hydrogen interactions. We thought that taking into account the structural features of the 14-membered macrolide antibiotics studied and on the basis of the mentioned observation [5a,5b] by measuring the spin-lattice relaxation times, it might be possible to differentiate at least some of the methyl resonances of the compounds investigated.

Carbon-13 spin-lattice relaxation times were measured as indicated previously [6] for erythromycin-A , erythromycin-B , oleandomycin , lankamycin 1 and picromycin [7]. The complete set of measured T_1 values for lankamycin 1 is shown in fig. 1. while Table 1 indicates the average ^{13}C N.M.R. NT_1 data for the CH and CH_2 type carbon atoms of the compounds examined.

From the inspection of the data shown in fig. 1. it is clear that in the case of lankamycin, longer relaxation times do not seem to be associated simply with a higher number of 1,3-diaxial methyl-hydrogen interactions. Probably other presently unknown

factors influence also the T_1 values or slight deviations from the equatorial and axial configurations are at the origin of the unexpected results.Using the methyl signal assignments of Nourse and Roberts [2c] the relaxation times are indicated in square brackets in fig. 1. and the agreement in this case with the observation [5a],[5b] that a higher number of 1,3-diaxial interactions results in a longer T_1 value is not better than with our δ-values.The conclusions are also negative in this respect in the case of the other macrolide antibiotics investigated.Clearly, further work will be necessary in order to carry out a one to one basis assignment for the methyl resonances of these compounds.

TABLE 1.

Average ^{13}C NT_1 data (in sec) for 14-membered macrolide antibiotics [a],[b]

Carbon type (CH + CH_2)	erythromycin A	erythromycin B	oleandomycin	lankamycin	picromycin
macrolide ring	0.34	0.34	0.32	0.33	0.84
desosamine	0.40	0.40	0.42	-	1.03
cladinose	0.40	0.37	-	-	-
oleandrose	-	-	0.41	-	-
chalcose	-	-	-	0.47	-
arcanose 4-O-Ac	-	-	-	0.37	-

a. ^{13}C N.M.R. spectra were recorded with 8K points in the time-domain on a Varian XL-100-15 F.T. spectrometer equipped with a Varian 620/1 computer.All the measurements were carried out in 0.2 M $CDCl_3$ solution at 36°C.For erythromycin-B the concentration and temperature were respectively 0.4 M and 58°.

b. For structural details of the compounds investigated see reference 2c.

It is of interest to note that in the case of the 14-membered macrolide antibiotics, the desosamine $N(CH_3)_2$ carbons do not exhibit significantly higher T_1 values than the C-6 methyl carbon directly attached to the pyranose ring.Similar observations were made but they were not reported [6] in connection with the mycaminose $N(CH_3)_2$ carbons of 16-membered macrolide antibiotics.

As far as the C-13 side chain carbon resonances of the aglycone of lankamycin

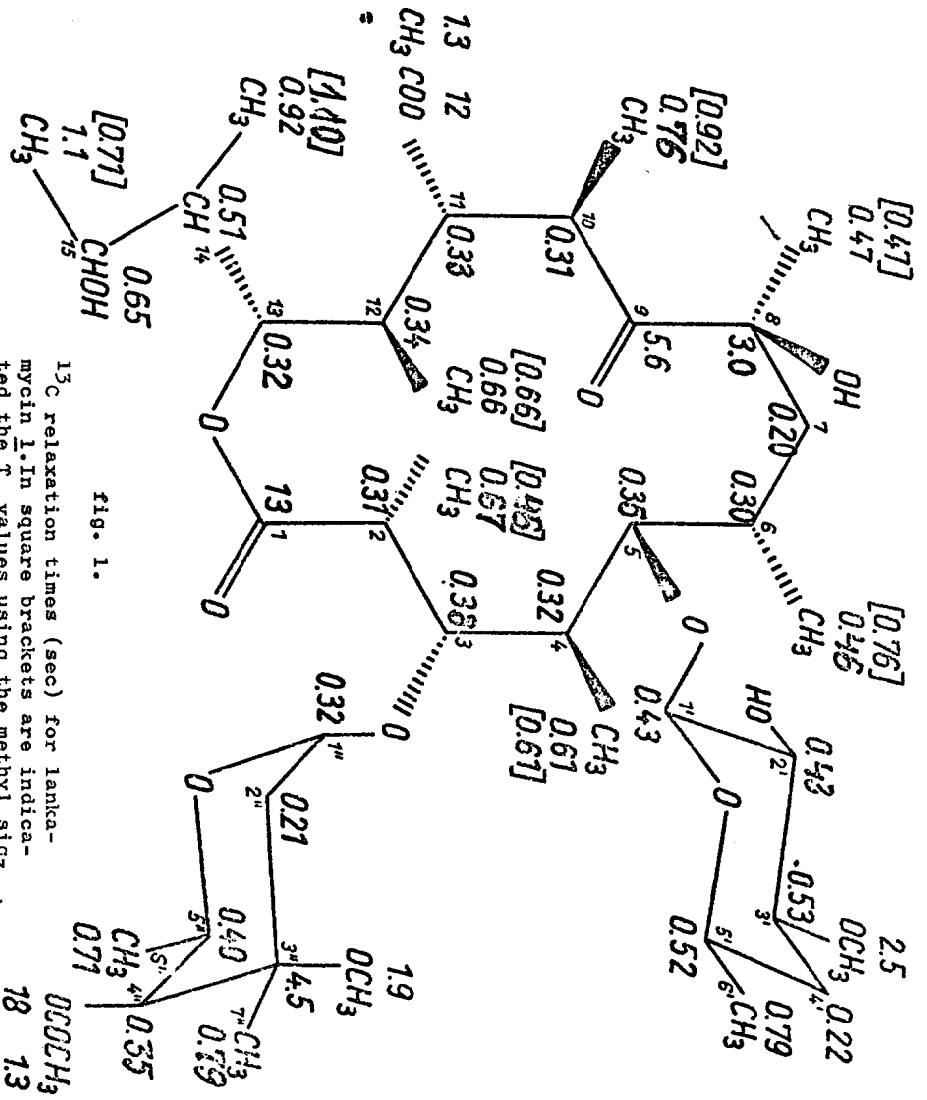

fig. 1.

^{13}C relaxation times (sec) for lankamycin 1.In square brackets are indicated the T_1 values using the methyl signal(2c) nal assignments of Nourse and Roberts The square bracket T_1 values for C-4Me, C-6Me and C-12Me were arbitrarily attributed since the corresponding carbons gave one signal in the american author's paper.

and the sugar signals of this antibiotic are concerned, our study helps to clarify the situation.Among the methine signals, as expected, C-15 shows the longest T_1 value , followed by C-14 and among the C-methyls C-15Me relaxes the slowest **followed by C-14Me. Furthermore** , the previously left ambiguity [2b] between some oxymethine signals (C-11 , C-13 , C-15 , C-2' and C-4") can be solved.Relaxation times of the carbohydrate carbons (chalcose and arcanose) are longer than those of the aglycone carbons in agreement with our results on 16-membered macrolide antibiotics [6].This important re-

sult seems to be general as it is illustrated by all the macrolide antibiotics studied during this work (see Table 1).As a consequence, it might be possible to differentiate the sugar resonances of the macrolide antibiotics from the aglycone signals without spectral comparison between the natural product and its carbohydrate components [8]. Final assignments, except for some C-methyl resonances, are presented for lankamycin 1 in Table 2.These assignments are in good agreement with the ^{13}C N.M.R. spectrum ($CDCl_3$) of the model methyl α-D-arcanoside recently synthesized in our laboratory [9].(Table 2)

TABLE 2.

^{13}C chemical shifts for lankamycin 1 [a.] and for the appropriate O-methyl derivatives of its sugar components [10] .The prime symbol is applied for convenience to the chalcose and the double prime symbol to the arcanose carbons [b.c.].

C-1	176.8	C-13	71.2	C-1'	102.4	methyl β-D-chalcoside	104.0	C-1"	96.6	methyl α-D-arcanoside	98.2
C-2	45.0	C-14	42.8	C-2'	75.6		74.7	C-2"	31.0		32.9
C-3	77.7	C-15	69.1	C-3'	80.4		80.2	C-3"	72.8		72.9
C-4	44.2	C-2Me	19.0[e.]	C-4'	37.4		37.4	C-4"	73.1		72.9
C-5	84.7	C-4Me	9.8	C-5'	67.4		68.0	C-5"	62.7		61.5
C-6	34.1	C-6Me	14.5[e.]	C-6'	21.0		21.0	C-6"	16.8		16.8
C-7	39.2	C-8Me	27.1	OCH_3	57.0		56.9	C-7"	21.0		20.9
C-8	80.1	C-10Me	10.4[e.]	OCH_3	-		56.9	OCH_3	49.3		49.5
C-9	214.5[d.]	C-12Me	10.2					OCH_3	-		55.4
C-10	39.4	C-14Me	11.2								
C-11	74.1	C-15Me	19.7								
C-12	38.3[d.]										

a. the lankamycin chemical shifts are slightly different (± 0.2 ppm) from those previously reported [2b] .This is due to the improved resolution obtained in the present work.

b. acetate carbons for lankamycin : 170.8 , 170.2 , 20.9 and 20.9

c. acetate carbons for methyl α-D-arcanoside : 170.6 , 21.0

d. and e. assignments for these signals may be reversed.

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