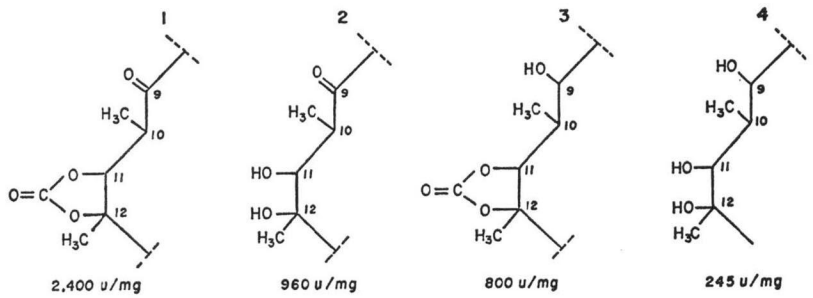
A C-13 RELAXATION STUDY ON ERYTHROMYCIN A CYCLIC 11,12-CARBONATE

Sir:

The erythromycin A cyclic 11,12-carbonate (**1**) is a compound with a pronounced antibacterial activity. Three functional groups are present which are playing a key role in the appearance of the antibacterial activity arising from the binding of erythromycin antibiotics to the 50S subunit of prokaryotic ribosome. These groups are the carbonyl function at C-9, the hydroxyl at C-2′ and dimethylamine moiety at C-3′. Comparison of the biological potency values,* as given below for **1**, its parent antibiotic erythromycin A (**2**), 9-dihydroerythromycin A 11,12-carbonate (**3**) and its parent 9-dihydroerythromycin A (**4**), both prepared according to ref. 1), showed that the introduction of the carbonate carbonyl group results in a pronounced increasing of the antibacterial activity.

The mechanism of action of **1** is similar to that of **2**[2].

The affinity of **1** for ribosomes must depend not only on the presence of the four above mentioned functions but also on the shape of the molecule in solution. The use of C-13 n.m.r. relaxation times may be mostly advantageous for the analyses of the shape and mobility of the molecule. Such measurements reported below for **1** enable us to discuss some aspects for its binding affinity to ribosomes which seem to be in common with all nonpolyenic macrolide antibiotics containing a desosamine moiety as a substituent.



The sample of **1** used in the present measurements represented a pure hemiacetal form. Note that in aqueous media the hydroxyketone tautomer will appear[3] lowering the rigidity of the C-6 ~ C-9 fragment to the aglycone moiety. The presence of the five membered carbonate ring at C-11 and C-12 in **1** enhances the rigidity in comparison to **2**.

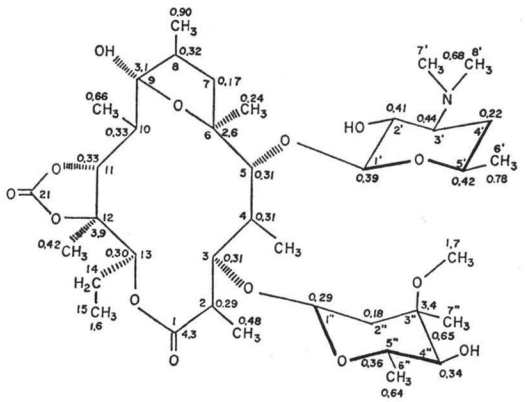
C-13 relaxation times in seconds for **1** are represented in the Fig. 1, taken at 60°C in 30% v/v $CDCl_3$ solution using an XL-100-FT-15 spectrometer (25.16 MHz) and the inversion-recovery method. Statistical errors are better than 5%.

The carbon resonance assignments are given in Table 1 as a result of detailed selective hetero decoupling measurements and the analysis of the both proton spectra at 100 and 352 MHz.

T_1 values of nonprotonated carbons in the macrolide ring have nearly identical (0.32 sec.) values clearly indicating the approximately isotropic motion[4] of the molecule in the solution and its rigidity. The validity of the dipole dipole relaxation mechanism is shown *e.g.* in the case of the C-7 methylene group its T_1 having a half of the above value.

Monoprotonated carbons of the aglycone, desosaminyl and cladinosyl rings give the average NT_1 values of 0.32, 0.42 and 0.34 sec. There is consequently a significant increase in motional

Fig. 1. Carbon-13 relaxation times of erythromycin A cyclic 11,12-carbonate (sec.).



* Average results of assays using cylinder-plate method and *Bacillus pumilus* NCTC 8241, carried out by Dr. D. Rośnik-Kamińska, Institute of Pharmaceutical Industry, Warszawa.

freedom only in case of the desosaminyl ring.

Methyl groups attached directly to the aglycone show T_1-s from 0.90 sec. down to 0.24 sec., free rotation corresponds to 0.96 sec. (three times the average NT_1) according to the DD mechanism. Its validity has been proved by NOE measurements. Various asymmetric steric compression effects may cause the observed hindrance in methyl rotation, the lowest T_1-s belong to CH_3 groups attached to quaternary carbons having also one O-atom bonded. The ethyl group at C-13 moves freely around both bonds involved.

Methyl groups attached to the aminosugar moiety have less than the maximal $3NT_1$ value. The fact that the T_1 value measured for the $N(CH_3)_2$ group is near to the $3NT_1$ of the desosamine ring clearly demonstrates the relative rigidity of the dimethylamino group with respect of the sugar frame (the C-3′ -N axis is not a "free" axis for rotation), stabilizing in this fragment the shape of the molecule, probably as required for the interaction with the bacterial ribosome. The flexibility of glycosidic linkage between C-5 and C-1′ is due to the free rotation around the C-5 -O and/or O- C-1′ bonds. This was also suggested by NOURSE and ROBERTS[5] for erythromycin. The flexibility of the discussed glycosidic linkage together with the defined position of the dimethylamino group with respect to desosaminyl ring point out the possible role of the dimethylamino group as anchoring the antibiotic molecule to the reacting element of the ribosome binding site thus initiating complexation with the ribosomal surface. The significant negative effect of 2′-hydroxyl esterification on the antibacterial activity can be correlated with the diminished ability of the dimethylamino group to anchor the antibiotic molecule to the ribosome binding site. This could be predicted by a direct steric hindrance of the ester grouping or by the inhibition by the same grouping of free rotation on the glycosidic linkage between C-5 and C-1′. The role of the carbonyl groups seems to be connected with the formation of hydrogen bonds.

The same relative rigidity of the dimethylamino group with respect to the aminosugar moiety was found previously on studying carbon-13 spin-lattice relaxation times for erythromycin A, erythromycin B and oleandomycin[6] as well as for 16-membered aglycone macrolides[7].

Table 1. Carbon-13 chemical shifts of erythromycin 11,12-carbonate referred to TMS (i)

Carbon	δ^{TMS}_{ppm}	Carbon	δ^{TMS}_{ppm}
1	177.8	2	41.7
9	107.5	7′+8′	40.5
1′	103.2	7	40.1
1″	95.7	4	38.5
12	86.6	10	36.8
6	86.2	2″	35.2
3	83.1	4′	28.8
5	82.7	6 Me	26.5
13	78.4	14	22.8
4″	78.3	7″	21.7
11	76.1	6′	21.3
3″	73.2	6″	18.3
2′	70.5	12 Me	16.8
5′	69.7	2 Me	13.0
3′	66.2	8 Me	12.7
5″	65.6	10 Me	12.6
O Me	49.6	4 Me	12.2
8	43.9	15 Me	10.5

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