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CARBON-13 N.M.R. SPECTRAL ANALYSIS AND SPIN-LATTICE RELAXATION TIMES OF THE ANTIBIOTIC LINCOMYCIN AND RELATED COMPOUNDS (1).

S. MIZSAK and G. SLOMP
(Research Laboratories of the Upjohn Company, Kalamazoo, Michigan 49001, USA)
A. NESZMELYI
(The Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Budapest , Pusztaszeri ut , Hungary)
S. D. GERO and G. LUKACS*
(Institut de Chimie des Substances Naturelles du CNRS., 91190-Gif-sur-Yvette France)

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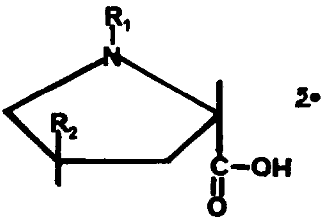
The antibiotic lincomycin, produced by Streptomyces lincolnensis, is known to be effective against gram-positive bacteria (2).Chemical modification of the lincomycin molecule has produced a series of analogs with significantly different potency than lincomycin (3).A possible explanation for these differences, based on steric and electronic effects, has been proposed by B.V. Cheney et al. (4).In their analysis the lowest-energy conformations were approximated by quantum mechanical calculations.Proton N.M.R. spectra of lincomycin and some related compounds have been analyzed and reported previously (5).

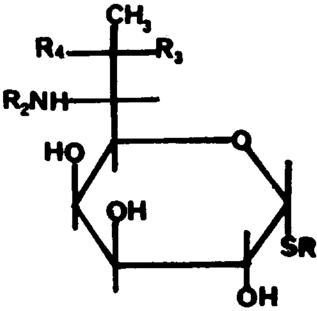
In this study we have examined the ^{13}C N.M.R. spectra of lincomycin • HCl 8 and a few related compounds as well as some simple components of the antibiotic.Furthermore, we have measured carbon-13 spin-lattice relaxation times for lincomycin • HCl 8 and clindamycin • HCl 11 in the hope of obtaining information about the solution behaviour of these biologically important materials.

Noise decoupled ^{13}C N.M.R. spectra were recorded (6) for compounds from 2 to 14 and the chemical shifts are given in the Table.The signal assignments are based on spectral comparison of the structurally related compounds studied, on chemical shift rules (7), on specific proton decoupling and single frequency off-resonance decoupling experiments and on spin-spin coupling with the phosphorus atom of 9 and 12.



1 $R_1 = H$; $R_2 = H$
2•HCl $R_1 = CH_3$; $R_2 = C_3H_7$-n

3 R_1=CH_3;R_2=$COCH_3$;R_3=H;R_4=OH
4 R_1=CH_3;R_2=$COCH_3$;R_3=OH;R_4=H
5•HCl R_1=CH_3;R_2=H ;R_3=Cl;R_4=H
6 R_1=CH_3;R_2=H ;R_3=Cl;R_4=H
7 R_1=CH_2CH_2OH;R_2=H;R_3=H;R_4=OCH_3

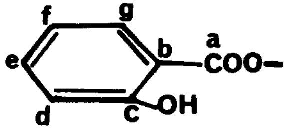


TABLE

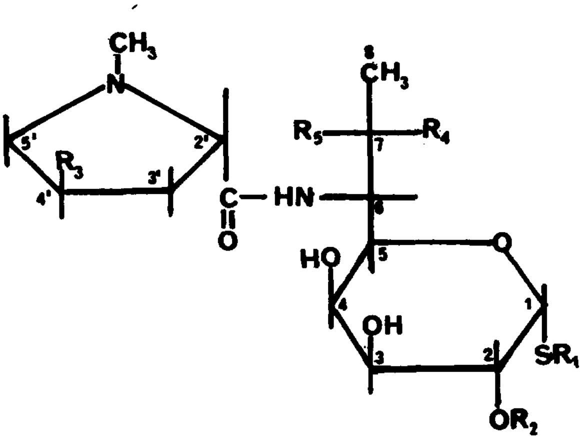
^{13}C chemical shifts

	1[b]	2	3	4	5	6	7	8	9[d]	10	11	12[d]	13	14[c]
C-1	-	-	89.0	88.2	87.5	87.3	87.0	89.2	87.5	88.3	88.8	87.0	87.9	88.3
C-2	-	-	68.7	68.8	66.6	67.4	70.7[a]	68.8	72.7	68.8	68.4	72.5	70.0[a]	70.3[a]
C-3	-	-	71.3	71.3	69.9	71.4	71.5	71.4	70.7	71.4	71.3	70.5	71.3	71.3
C-4	-	-	69.3	69.4	67.5	67.6	68.3[a]	69.5	69.4	69.5[a]	69.2	69.1	70.0[a]	69.9[a]
C-5	-	-	70.6	69.5	69.3	70.4	67.8[a]	70.0	70.0	69.0	68.9	68.9	69.3[a]	69.3[a]
C-6	-	-	53.9	53.8	54.4	54.4	51.4	54.9	54.9	54.5	54.0	53.9	50.6	50.8
C-7	-	-	67.6	65.7	57.3	60.6	76.5	67.4	67.4	65.6	59.0	58.7	76.8	76.7
C-8	-	-	16.9	20.6	22.3	22.4	12.7	17.2	17.3	20.8	23.0	22.4	14.7	14.7
S-CH$_3$	-	-	14.0	13.3	12.4	12.3	-	14.2	14.1	13.5	13.8	13.4	-	-
C-2'	62.1	65.8	-	-	-	-	-	69.5	69.4	69.3[a]	69.8	69.7	68.5[a]	68.5[a]
C-3'	29.8	34.4	-	-	-	-	-	36.4	36.3	36.6	36.4	36.4	30.7	30.8
C-4'	24.6	35.1	-	-	-	-	-	37.4	37.4	37.5	37.5	37.3	23.9	23.9
C-5'	47.5	60.5	-	-	-	-	-	62.4	62.4	62.3	62.1	62.0	57.6	57.7
α-CH$_2$	-	33.4	-	-	-	-	-	35.0	35.0	35.0	34.5	34.4	-	-
β-CH$_2$	-	20.5	-	-	-	-	-	21.5	21.5	21.5	21.5	21.2	-	-
γ-CH$_3$	-	13.8	-	-	-	-	-	14.3	14.3	14.4	14.3	14.0	-	-
N-CH$_3$	-	39.6	-	-	-	-	-	41.8	41.7	41.8	42.1	41.5	41.9	41.9
C=O	175.3	169.1	175.1	175.3	-	-	-	170.1	169.8	169.9	169.8	169.6	169.4	169.1
Ac.CH$_3$	-	-	23.1	23.0	-	-	-	-	-	-	-	-	-	-
S-CH$_2$	-	-	-	-	-	-	32.6	-	-	-	-	-	33.8	30.2
O-CH$_2$	-	-	-	-	-	-	61.0	-	-	-	-	-	61.8	65.5
O-CH$_3$	-	-	-	-	-	-	55.7	-	-	-	-	-	57.1	56.9

[a] signal assignments within a vertical column may be reversed. [b] chemical shifts according to L.F. Johnson and W.C. Jankowski, "Carbon-13 NMR spectra" , John Wiley and Sons , New-York , N.Y. 1972. [c] sp^2 carbons for celesticetin • HCl 14 : (a) 169.9 ; (c) 160.8 ; (e) 136.6 ; (g) 131.1 ; (f) or (d) 120.4 ; (d) or (f) 117.9 and (b) 112.8



[d] spin coupling of less than 10 Hz is observed with the phosphorus atom for carbons-1 , -2 and -3.



8 • HCl $R_1=CH_3;R_2=H;R_3=C_3H_7\text{-n};R_4=H;R_5=OH$
9 • HCl $R_1=CH_3;R_2=PO_3H_3;R_3=C_3H_7\text{-n};R_4=H;R_5=OH$
10 • HCl $R_1=CH_3;R_2=H;R_3=C_3H_7\text{-n};R_4=OH;R_5=H$
11 • HCl $R_1=CH_3;R_2=H;R_3=C_3H_7\text{-n};R_4=Cl;R_5=H$
12 • HCl $R_1=CH_3;R_2=PO_3H_3;R_3=C_3H_7\text{-n};R_4=Cl;R_5=H$
13 • HCl $R_1=CH_2CH_2OH;R_2=H;R_3=H;R_4=H;R_5=OCH_3$
14 • HCl $R_1=CH_2CH_2OCOC_6H_4OH;R_2=H;R_3=H;R_4=H;R_5=OCH_3$

Carbon-13 spin-lattice relaxation times (8) for lincomycin•HCl 8 and clindamycin•HCl 11 are shown on Figure 1.Examination of the T_1 values for the molecules under investigation yields the following conclusions (9):

a. the similarity of the T_1 values for the pyranose ring carbons indicates isotropical tumbling of this moiety without preferred axis of rotation.

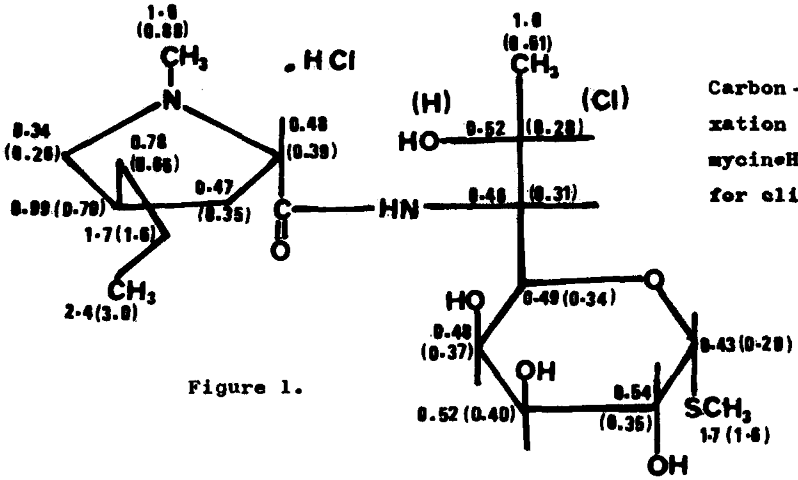
b. the motion of C-6 and C-7 is isotropic with the sugar ring as evidenced by the essentially identical T_1 values with those of the pyranose methine carbons (no free rotation around the C-6/C-7 axis).This rigidity was noted earlier (4),(5).

c. the pyrrolidine ring is characterized by an increased mobility with respect to the sugar moiety;NT_1 values are almost twice higher for the five-membered ring than for the six-membered ring (N is the number of the directly bonded hydrogens).The NT_1 value for C-2' is markedly lower than the average NT_1 value for the pyrrolidine ring suggesting that C-2' is on a preferred axis of rotation (9).

d. considerable free rotation is experienced by the methyl groups (9) (C-methyl N-methyl and S-methyl) and the C-4' side chain carbons.NT_1 (CH_3 or C-4' side chain carbons) $\gg$ NT_1 (pyranose ring carbons, pyrrolidine ring carbons, C-6 and C-7).

The only major difference between the solution mobility of lincomycin•HCl 8 and clindamycin•HCl 11 is shown by the shorter T_1 values measured for the chlorine containing compound.This difference may be associated with the presence of the heavy chlorine atom.

As the relaxation behaviour of the two compounds examined is similar in all other respects, this is evidence for the previously proposed (4) conformational similarity of lincomycin and clindamycin.The chemical shift data of the Table indicates also the conformational identity of lincomycin•HCl <u>8</u> and clindamycin•HCl <u>11</u>.



Carbon-13 spin-lattice relaxation times (sec) for lincomycin•HCl <u>8</u> and in brackets for clindamycin•HCl <u>11</u>.

Figure 1.

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6. Carbon-13 N.M.R. spectra were measured in D_2O solution containing 1,4-dioxane on a Varian XL-100-15 F.T. spectrometer at 25.15 MHz for <u>3</u> , <u>4</u> , <u>8</u> , <u>9</u> , <u>10</u> , <u>11</u> , <u>12</u>, <u>13</u> and <u>14</u>.All chemical shifts are given in ppm relative to TMS using the relationship: δ TMS = δ 1,4-dioxane + 67.4 ppm.DMSO-d_6 was used as a solvent containing TMS for compounds <u>2</u> , <u>5</u> , <u>6</u> and <u>7</u>.
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