Lincomycin. IV. Nuclear Magnetic Resonance Studies on the Structure of Lincomycin, Its Degradation Products, and Some Analogs[1]

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Abstract: The contributions of nmr spectroscopy to the determination of the structure of lincomycin are reported. The amino acid portion was partially identified by nmr by comparison with synthetic samples. The structure of the sugar portion was independently determined by analysis of nmr spectra. The observation of an unusual intramolecular hydrogen bonding yielded the configuration of the sugar by conformational analysis. The spectra of some analogs and degradation products are correlated with their structures and conformations.

The structure of lincomycin,[2] an important new antibiotic with activity against gram-positive organisms, was the subject of a brief communication from these laboratories.[3] The present paper describes the nmr studies on lincomycin, some degradation products, and some analogs.

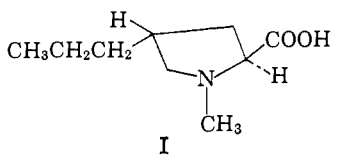
The nmr spectrum of lincomycin hydrochloride ($C_{18}H_{33}N_2O_6SCl$) (Figure 1) is complex, containing many superimposed multiplets which were difficult to factor. There are several prominent signals which can be interpreted, but the severe overlapping, especially in the carbinol hydrogen region, makes complete interpretation impossible. The spectrum observed at 100 Mc[4] is not greatly different, indicating the close proximity of the shifts of the carbinol hydrogens. Attention was therefore turned to degradation products and derivatives.

Chemical experiments[5] showed that the molecule could be hydrolyzed at the amide linkage into an amino acid ($C_9H_{17}NO_2$) (I) and an amino thio sugar (methyl thiolincosaminide, MTL) ($C_9H_{19}NO_5S$) (II). Various aspects of the structures of these two compounds were determined by nmr, and when combined with chemical information the complete structure for the antibiotic was determined.

Amino Acid Portion. The nmr spectrum of the amino acid moiety (I) is shown in Figure 2. The material was thought to be a propyl-substituted hygric acid by comparison with known L-hygric acid.[6] The location and configuration of the alkyl group could not be determined from the spectrum because the 2- and 5-hydrogen multiplets could not be factored. The 2-hydrogen multiplet was an unsymmetrical three-line pattern with some additional absorptions between the three peaks. It could be rationalized as several partly superimposed doublets, each arising from the 2-hydrogen of molecules having a different ring conformation or different N-methyl configurations. Similar reasoning might also apply to the 5β-hydrogen resonance. Subsequent experiments at increased temperature[7] showed that at 100° the 2-hydrogen resonance collapsed to a regular triplet (J = 9.0 cps) and the 5β-hydrogen resonance became a doublet of doublets (J = 5 and 10 cps). The original spectrum was regenerated when the sample returned to room temperature. Abraham and McLauchlan indeed found such ring buckling in the closely related *cis*- and *trans*-4-hydroxy-L-prolines[8] and further concluded that the latter was a mixture of conformers.[8c]

A synthetic sample of D-*cis*-4-propylhygric acid gave a spectrum which appeared to represent only one conformer (Figure 3). It was noted from the nmr spectrum that a sample of the unknown hygric acid was unexpectedly isomerized to the *cis*-4-propylhygric acid. This focused attention on the possibility that the unknown acid was the 4-*trans* isomer and that it had epimerized at C-2 in this experiment.

The structure of the amino acid was proved as L-*trans*-4-*n*-propylhygric acid (I) by synthesis and degradation.[9]



I

The analogous *trans*-4-methylproline reported by Abraham and co-workers[8c] gave an nmr spectrum uncomplicated by conformers as did also the amide of *trans*-4-propylhygric acid hydrochloride and the quaternary analog *trans*-4-propylstachydrine (the betaine of I). L-Proline gave a spectrum similar to that of I with conformational complications. This was not noted by Abraham, *et al.*,[8c] in their analysis of this spectrum. We plan to investigate this isomerism further.

Amino Thio Sugar Portion. The nmr spectrum of MTL (II) in deuterium oxide (Figure 4) showed absorptions for six kinds of hydrogen, as follows: (a) a doublet

(1) This material was presented in part at the 148th National Meeting of the American Chemical Society, Chicago, Ill., Aug–Sept 1964, Abstracts, p 37S.

(2) See footnotes 1–5 of ref 3.

(3) H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. Schroeder, G. Slomp, and R. R. Herr, *J. Am. Chem. Soc.*, **86**, 4223 (1964).

(4) We are grateful to Dr. Leroy Johnson of Varian Associates for this determination.

(5) W. Schroeder, B. Bannister, and H. Hoeksema, *J. Am. Chem. Soc.*, **89**, 2448 (1967).

(6) R. R. Herr and G. Slomp, *ibid.*, **89**, 2444 (1967).

(7) We thank Mr. C. R. Joller and his staff at the Pittsburgh Service Center of Varian Associates for assistance in these measurements.

(8) (a) R. J. Abraham and K. A. McLauchlan, *Mol. Phys.*, **5**, 195 (1962); (b) R. J. Abraham and K. A. McLauchlan, *ibid.*, **5**, 513 (1962); (c) R. J. Abraham, K. A. McLauchlan, S. Dalby, G. W. Kenner, and R. C. Sheppard, *Nature*, **192**, 1150 (1961).

(9) B. J. Magerlein, R. D. Birkenmeyer, R. R. Herr, and F. Kagan, *J. Am. Chem. Soc.*, **89**, 2459 (1967).

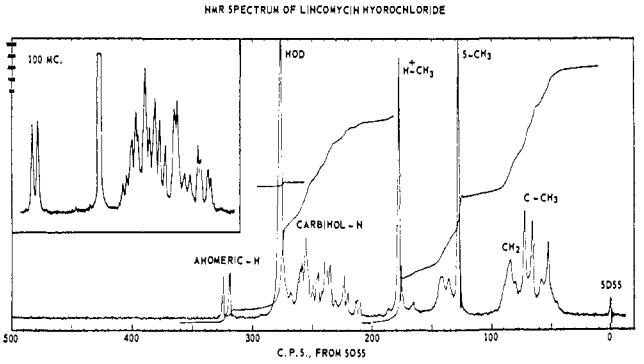


Figure 1. Nmr spectrum of lincomycin hydrochloride.

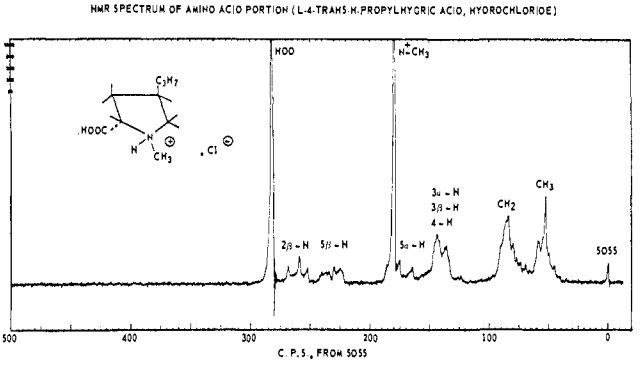


Figure 2. Nmr spectrum of amino acid portion (L-4-*trans*-*n*-propylhygric acid hydrochloride) in D_2O.

of area 3 at 68.0 cps,[10] J = 6.5 cps, indicative[11] of a carbinol methyl group coupled to only one neighboring hydrogen; (b) a singlet of area 3 at 127 cps, assigned to the S-methyl group; (c) a doublet of doublets of area 1 at 188.5 cps, J = 10.5 and 3.0 cps, indicative of a carbamine hydrogen coupled differently to two neighbors; (d) a group of resonance lines with a total area of 5, lying between 212 and 256 cps, arising from the carbinol hydrogens of the sugar; (e) the HOD line at 276 cps, containing the resonances of six exchangeable hydrogens on oxygen and nitrogen; and (f) a doublet of area 1 at 320 cps, J = 5.5 cps, attributed to the anomeric hydrogen of the sugar. The multiplets are expanded for further study in Figure 5.

The nmr spectrum of MTL contained evidence that the molecule was a sugar. The presence of an anomeric hydrogen signal suggested an aldose, but aldoses are usually mixtures of many isomeric forms. The absence of mixture characteristics and the presence of a methylthio group suggested it was a methyl thioglycoside. The size of all the couplings and the general lack of sharpness suggested the rigidity of an aldopyranoside rather than a furanoside.[12]

The spectrum was empirically factored as much as possible using a dividers and making use where possible of the line shapes of the simple multiplets to evaluate J/δ frequency and to determine δ frequency (see Figure 5). The empirical factoring was subsequently confirmed

(10) Spectra were calibrated in cps units at 60 Mc, downfield from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (SDSS) [G.V.D. Tiers and R. I. Coon, *J. Org. Chem.*, **26**, 2097 (1961)].

(11) K. Nukada, *et al.*, *Anal. Chem.*, **35**, 1892 (1963).

(12) Furanosides owing to their rapid ring conversion often give sharp spectra, and most of the coupling constants are of an averaged magnitude which is about 3 cps.

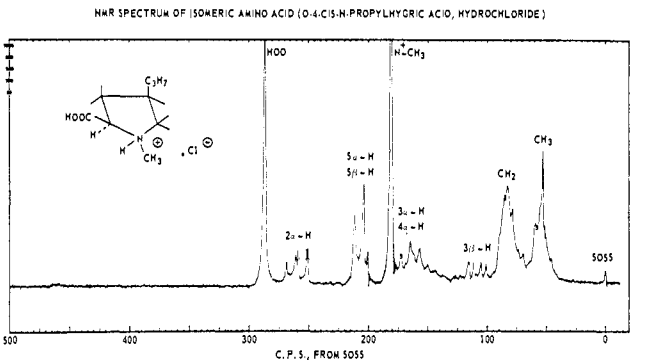


Figure 3. Nmr spectrum of isomeric amino acid (D-4-*cis*-*n*-propylhygric acid hydrochloride) in D_2O.

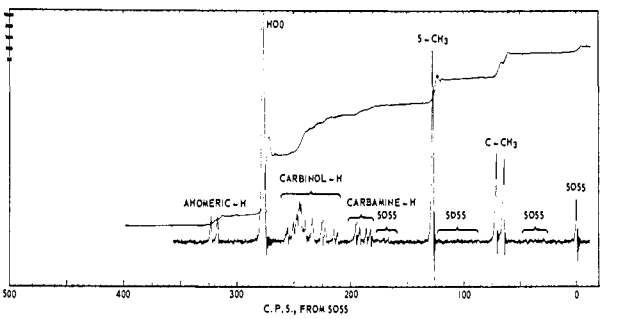


Figure 4. Nmr spectrum of methyl thiolincosaminide in D_2O.

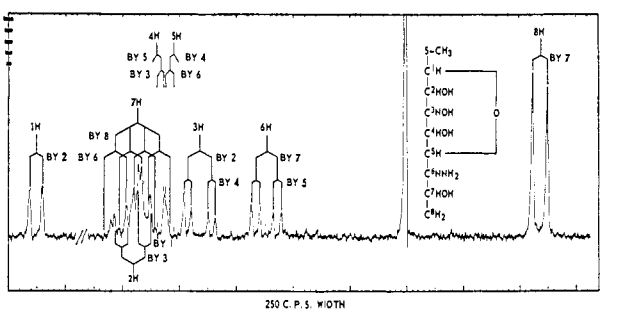


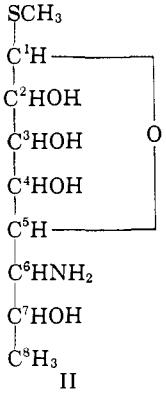
Figure 5. Portions of the nmr spectrum of methyl thiolincosaminide in D_2O.

by spin-decoupling experiments.[7] The factored spectrum revealed the structure of the sugar. The anomeric hydrogen was observed in the spectrum as a doublet at 320 cps. It was coupled ($J_{1,2}$ = 5.5 cps) to one neighboring hydrogen at C-2. The absorption of this 2-H was found at 248 cps, in the carbinol hydrogen region, and C-2, therefore, bears one hydrogen and one hydroxyl group. The 2-H absorption was actually a doublet of doublets and in addition to the coupling to 1-H it was further split ($J_{2,3}$ = 10.5 cps) by one hydrogen at C-3. The absorption of this 3-H was accordingly found at 218 cps, in the carbinol hydrogen region. Thus, C-3 bears one hydrogen and one hydroxyl group. The 3-hydrogen absorption was actually a doublet of doublets with the same $J_{2,3}$ but with a second coupling ($J_{3,4}$ = 3.0 cps) to one hydrogen at C-4. The absorption of this 4-H could be extracted from the spectrum only by spin-decoupling experiments,[7] and it was found at 240 cps, in the carbinol hydrogen region. Thus, C-4 bears one hydrogen and one hydroxyl group. Since the 4-hydrogen multiplet could not be factored, attention was turned to the other end of the molecule.

The methyl group embodying the 8-hydrogens[13] absorbed as a doublet at 68 cps. These hydrogens were therefore coupled to one neighboring hydrogen at C-7. The absorption of this 7-H was found at 245 cps, again in the carbinol hydrogen region, and C-7, therefore, bears one hydrogen and one hydroxyl group. The 7-H absorption was actually a doublet of quartets and in addition to the coupling to the three hydrogens at C-8 it was further split ($J_{6,7}$ = 9.25 cps) by one hydrogen at C-6. The absorption of this 6-H was accordingly found at 188 cps, in the carbamine hydrogen region. Thus, C-6 bears one hydrogen and one amine group. This 6-hydrogen absorption was actually a doublet of doublets with the same $J_{6,7}$ but with a second coupling ($J_{5,6}$ = 3.75 cps) to one neighboring hydrogen at C-5. The absorption of this 5-H could be extracted from the spectrum only by spin decoupling[7] and it was observed at 228 cps, in the carbinol hydrogen region, but again this multiplet could not be factored. Thus, C-5 bears one hydrogen and one oxygen substituent which in this case must be the pyran ring (*vide supra*).

The similarity of the shift of the 4- and 5-hydrogens apparently resulted in a very closely coupled multiplet which was not factored by empirical procedures. Such a multiplet would have an intense center at about 234 cps as shown in Figure 5, but the outlying portions would be weak and would not be seen in the spectrum.

Since all the peaks in the spectrum have been accounted for and all the alloted hydrogens have been used, it remains to connect C-4 and C-5 to yield structure II for this sugar. This analysis independently



II

confirmed earlier chemical evidence[6] that MTL was a thiomethyl pyranoside of an aminodideoxyoctose but disagreed in the position of the NH_2 substituent.[14]

The data so far obtained also provided some information regarding the conformation and configuration of MTL. In a pyranoside ring some deformation is possible, as for example in β-glucopyranose,[15] and the dihedral angles are not always exactly 60 or 180°. Even though the Karplus relationship does not yield *exact* dihedral angles from coupling constants,[16] the *magnitude* of the calculated angle does allow a distinction between neighboring hydrogens that are diaxial *vs.* those which are diequatorial or axial–equatorial. The observed coupling constants are, therefore, summarized in Table I, and the appropriate conformational relationships have been deduced from the Karplus angles.

Table I. Couplings[a] and Possible Conformations of the Sugar Hydrogens[b]

J_{xy}	Cps	Conformational relationship	—Dihedral angle, deg— Calcd	Found[c]
1,2	5.5	*ea* or ee[d]	*33* or 127	60
2,3	10.5	*aa*	180	161
3,4	3.0	*ae* or ee	*50* or 112	42
4,5	?	?	?	60
5,6	3.75	*ae* or ee	*46* or 117	45
6,7	9.25	*aa*	150	163
7,8	6.5	Free rotation	...	...

[a] The coupling constants are simply first-order values obtained from the spacings in the spectra. Some of these approximate values may thus be in error by as much as 0.5 cps but this is not serious since exact fitting of angles was not sought. [b] The italic values were the ones that fit the final structure. [c] Observed in the Dreiding model of Ia. [d] a = axial, e = equatorial.

The configuration of the sugar can now be deduced from the dihedral angle data of Table I. Since the 2-H–3-H dihedral angle must be about 180°, these two hydrogens must be *trans* diaxial. A chair-formed pyranose ring was arbitrarily drawn in the D form and axial hydrogens were placed at carbons 2 and 3. Now if 2-H and 3-H are axial, the 1-H–2-H and the 3-H–4-H relationships cannot be equatorial–equatorial but must be equatorial–axial and axial–equatorial, respectively. Equatorial hydrogens were placed at carbons 1 and 4. Now the 4-H–5-H angle is not known because these multiplets could not be factored. However, one may assume that the alkyl chain at C-5 is equatorial because this is the more stable form which is reached by ring conversion between the two possible chair forms. Thus, the 5-H must be axial and the ring has the α-galactoside configuration.

It was noteworthy that the signals arising from the 6- and 7-hydrogens were not very sharp. This was observed by comparing their ringing with that from the methyls. Also, the coupling constants of the 5-, 6-, and 7-hydrogens were not about 7 cps as would be expected for a freely rotating side chain. It was therefore concluded that this chain was held rigid, in one preferred conformation. Steric effects observed from Catalin models did not seem to be serious enough to bring about this rigidity, but the opportunity for hydrogen bonding from the side chain to the ring in several different ways was clearly apparent from the Dreiding models. To adequately immobilize the side chain the 7-hydroxyl was suspected of involvement. Although several sites were possible, the chelate formed by bonding the 7- to the 4-hydroxyl group looked ideal in the models.[17] This chelate ring contained seven atoms.[17]

Further information on the configuration of the side chain was now obtained by conformational analysis. From the coupling constants (Table I) the 6-H–7-H angle must be about 150° and the 5-H–6-H angle about 46 or 117°. The eight possible side-chain conforma-

(13) The empirical formula and subsequent reasoning (*vide infra*) showed that the molecule terminated with C-8.

(14) The negative iodoform test[5] required the NH_2 group to be at C-7, but this test was subsequently shown to be anomalous.

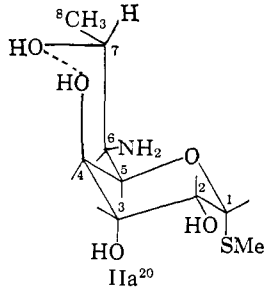
(15) R. W. Lenz and J. P. Heeshen, *J. Polymer Sci.*, **51**, 247 (1961).

(16) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2871 (1963).

(17) Hydrogen bonds vary in energy but are strongest when certain spatial requirements are met. The optimum OH–O distance in alcohols is about 2.74 A and collinearity is desirable.[18] Thus, intramolecular hydrogen bonds lead to various-sized rings, but those containing five to seven atoms are common.

(18) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman and Co., San Francisco, Calif., 1960, Chapters 5 and 7; J. D. Bernal in "Hydrogen Bonding," D. Hadzi and H. W. Thompson, Ed., Pergamon Press Inc., New York, N. Y., 1959, p 7.

tions allowed by these angle requirements were examined with the aid of Dreiding models and only that shown in IIa affords both the approximate required

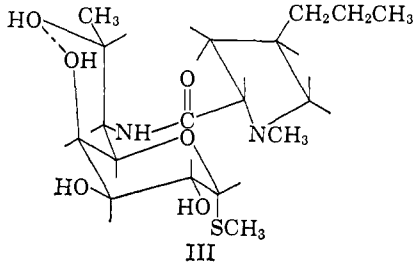


IIa[20]

angles and the possibility of hydrogen bonding from the 7-OH to the ring.[19] Thus, the analysis led to the conclusion that MTL was either IIa or its enantiomorph.

This structure, containing two *cis*-fused rings, both in the chain form, looked reasonable. The 8-methyl and 7-amine were both in the low-energy equatorial conformation. The foregoing reasoning was admittedly speculative at several points, but the results were completely confirmed and the D series was indicated by subsequent chemical degradations of MTL yielding D-galactose[5] from the pyranose portion, and D-*allo*-threonine[5,21] from the side-chain portion.

Reconstitution of the amide linkage yields III as the structure of lincomycin.



III

Resonance data obtained on some analogs and derivatives of MTL are collected in Table II. The glycoside configurations were determined from the nmr data. The spectrum (Figure 6) of 2-hydroxyethyl thiocelestosaminide (HTC) (IV), the sugar portion of celesticetin,[22] was identical with that of MTL after allowing for the replacement of the S-methyl absorbance by the A_2X_2 absorbance of the hydroxyethylthio substituent and the addition of a 7-methoxy signal,[23] thus confirming[22] that the sugar moieties of both antibiotics have the same configuration and showing that

(19) A second-choice structure had less desirable ring size and poorer agreement with observed angles but the same side-chain configuration as Ia. It had the 7-OH bonded to the ether oxygen to make a six-membered ring with an unusually long O–O distance. This model also required 5H–6H and 6H–7H angles to be 120° instead of 150 and 117° as observed. The rejection of this chelate was later confirmed when the 7-methoxy sugar was studied. It, too, showed the same hydrogen bonding; *vide infra*.[20]

(20) Since the exact location of the hydrogens in the hydrogen bond is not known, the structure is written in this noncommital form. It may be possible that both hydrogens are involved in a diamond arrangement with the two oxygens.[18]

(21) B. Bannister and H. Hoeksema, Abstracts, 148th National Meeting of the American Chemical Society, Chicago, Ill., Aug–Sept 1964, p 6P.

(22) H. Hoeksema, *J. Am. Chem. Soc.*, **86**, 4224 (1964).

(23) The 6-hydrogen of HTC absorbed 16 cps higher than that of MTL. This carbamine hydrogen could easily shift this much as a result of pH differences in these two solutions.

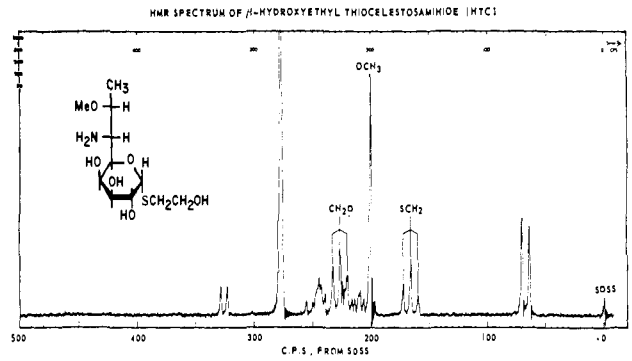
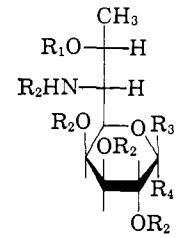


Figure 6. Nmr spectrum of β-hydroxyethyl thiocelestaminide in D_2O.

HTC must be the α anomer. The presence of a 7-methoxy group in HTC did not alter the chelation.

Compounds V, VI, VII, and VIII constitute two anomeric pairs of sugars and the glycoside configuration was deduced from the following observations.



II, $R_1 = R_2 = R_3 = H$; $R_4 = SCH_3$
IV, $R_1 = CH_3$; $R_2 = R_3 = H$; $R_4 = SCH_2CH_2OH$
V, $R_1 = R_2 = Ac$; $R_3 = SCH_3$; $R_4 = H$
VI, $R_1 = R_2 = Ac$; $R_3 = H$; $R_4 = SCH_3$
VII, $R_1 = R_2 = Ac$; $R_3 = OAc$; $R_4 = H$
VIII, $R_1 = R_2 = Ac$; $R_3 = H$; $R_4 = OAc$
IX, $R_1 = CH_3$; $R_2 = Ac$; $R_3 = OAc$; $R_4 = H$
X, $R_1 = CH_3$; $R_2 = Ac$; $R_3 = H$; $R_4 = SCH_2CH_2OAc$

The nmr spectra, summarized in Table II of the β anomers of MTL-pentaacetate (V) and lincosamine hexaacetate (VII), differed from the α anomers (VI and VIII) by showing 1-H resonances at lower frequency and $J_{1,2}$ couplings that were larger.[24] Since the 2-H in galactose is axial, the 1-H of the β isomer would be in a diaxial relationship to it, and this is in agreement with the 7.5- and 9-cps coupling constants.

Additional conclusions can be drawn from the 2-, 3-, and 5-H resonance frequencies. In the α anomers, the axial S or O atom at 1 is nearer the symmetrically located axial hydrogens at 3 and 5 than it is in the β anomers. This deshielding effect of the O or S would be expected to increase the 3- and 5-H resonance frequency, and this was indeed observed as assigned. The differences were 20, 20, 34, and 17 cps. One would accordingly expect the 2-H to resonate at higher frequency in the β anomers since the O or S atom is now slightly closer than in the α anomer. This was also observed on these pairs. The actual differences were 9 and 13 cps.

(24) In a study of the nmr of many pyranosides, Lemieux, *et al.*,[25] reported that in cases of rigid six-membered rings, equatorial hydrogens absorbed at a lower magnetic field (larger cps from TMS) than axial hydrogens did. Similar results have been observed in these laboratories. Further, it was noted that diaxial hydrogen neighbors showed a coupling constant of 5–10 cps and axial–equatorial or diequatorial hydrogen neighbors a coupling of 3–5 cps (the variation arises from some departure from perfect chair conformations[15]).

(25) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958); **82**, 6427 (1960).

Table II. Sugar Moiety Proton Absorbances[a] and Coupling Constants

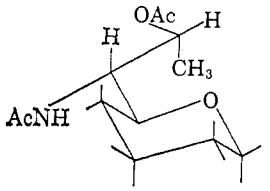
Material	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$[c]	$J_{4,5}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$
α-MTL[b] (II)	320	248[c]	218	240[c]	228[c]	188	245	68	5.5	10.5	3.0	...	3.75	9.25	6.5
α-HTC[b] (IV)	325	248[d]	220	240	225	204	245	67	5.5	10.0	2.5	...	3.0	9.5	6.5
β-MTL pentaacetate (V)	261	318	300	326	224	272	307	78	9.0	10.0	3.0	<1	9.5	3.0	7.0
α-MTL pentaacetate (VI)	339	305[d]	320[d]	326	258	277	306	77	4.75	...	...	<1	10.5	2.5	7.0
β-Lincosamine hexaacetate (VII)	344	322	305	326	231	277	307	73	7.5	10.0	2.5	≤2	10.0	3.0	7.0
α-Lincosamine hexaacetate (VIII)	383	313[d]	325[d]	329	248	272	306	73	2.0	10.0	2.5	<1	10.0	3.0	7.0
β-Celestosamine pentaacetate (IX)	343	321	307	327	238	268	210	71	7.0	10.0	3.0	<1	10.0	2.5	7.0
α-HTC[1] pentaacetate (X)	348	305[d]	315[d]	326	260	275	211	71	5.0	...	2.0	...	10.0	2.5	7.0
7-Chloro-7-deoxylincomycin (XV)	325	249	219	235	260	264	272	87	5.5	10.5	3.5	1	10.5	1.5	7.0

[a] II, IV, and XV were observed in D_2O with SDSS reference, others were in $CDCl_3$ with TMS reference. [b] MTL, methyl thiolincosaminide; HTC, 2-hydroxyethyl thiocelestosaminide. [c] Centers of absorbances located by spin-decoupling experiments. [d] Absorbances not factored completely, centers are approximated.

The celestosamine pentaacetate (IX) was judged to be β from the nmr data because of the very close similarity to that of β-lincosamine hexaacetate (VII) in all four of the above criteria. The fact that all the shifts and couplings in IX compared so closely to those of β-lincosamine hexaacetate (VII) confirms the conclusions that the two sugars are the same configuration and conformation.

The HTC pentaacetate sample X, when compared by nmr spectroscopy to the α- and β-MTL pentaacetates (VI and V), was judged to be the α anomer because of the high-frequency 1-H resonance, the small $J_{1,2}$, the high-frequency 3- and 5-H resonance, and the low-frequency 2-H resonance. Since this compound came from celesticetin with no possible inversion at C-1, the celesticetin precursor must have been the α anomer.

Inspection of Table II reveals that the coupling constants of the 5-, 6-, and 7-hydrogens in the spectra of the acetylated sugars as a group were now quite different from those found in the MTL and HTC spectra. It is apparent that the esterification of the OH groups destroyed the chelate ring and as a result the side chain shifted to a second preferred orientation VIa. In

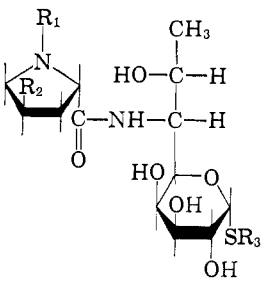


VIa

these acetylated molecules steric effects may well be the decisive factor. Catalin models indeed showed VI with the 5,6-hydrogens *trans* and the 6,7-hydrogens *gauche* to be a conformation with minimum steric interference among bulky acetyl groups.

Analogs. Many analogs of lincomycin have been studied by nmr. Extensive alterations of either the amino acid or sugar portions of lincomycin brought appropriate changes in the nmr spectrum. Minor modifications only altered the spectrum slightly. Thus, nmr served as a convenient tool for the identification of these analogs.

Alteration of the fermentation conditions and ingredients has produced several close analogs of lincomycin which were identified as XI–XIV.[26] When the R_1 substituent was hydrogen instead of methyl the N-



III, $R_1 = CH_3$; $R_2 = n\text{-}C_3H_7$; $R_3 = CH_3$
XI, $R_1 = CH_3$; $R_2 = C_2H_5$; $R_3 = CH_3$
XII, $R_1 = CH_3$; $R_2 = n\text{-}C_3H_7$; $R_3 = C_2H_5$
XIII, $R_1 = H$; $R_2 = n\text{-}C_3H_7$; $R_3 = CH_3$
XIV, $R_1 = H$; $R_2 = n\text{-}C_3H_7$; $R_3 = C_2H_5$

methyl singlet disappears from the spectrum and the new N-hydrogen appears with the exchangeable hydrogens. When R_1 was higher alkyl, the position and shape of the new absorptions from these alkyl groups were observed as expected.[27] The appropriate carbamine and terminal hydrogen frequencies are collected in Table III. It was noteworthy that the two methyls of

Table III. Characteristic Absorptions of N-Alkyl Analogs of Lincomycin Hydrochloride[a]

N substituent	Frequency, cps (J, cps) N-CH	C-CH₃
Methyl	179	
Ethyl	199 (7.5)	77 (7.5)
n-Propyl	193[b] (7.5)	57 (7.5)
Isopropyl	217[b] (6.5)	78, 80 (6.5)
n-Butyl	190[b] (6.5)	53 (7.0)

[a] D_2O was the solvent. [b] Unusually broad.

the isopropyl group experience slightly different shielding. It is proposed that this arises from restricted rotation of this isopropyl group.

When the R_2 substituent was an alkyl group other than propyl, very little change was observed in the spectrum compared to that of lincomycin hydrochloride. The area of the methylene absorption and the characteristics of the terminal methyl absorption varied as expected[27] for these substituents.

When R_2 was hydrogen or ethoxy, the spectra lacked the methylene hump and the methyl triplet. The ethoxy analog showed instead a carbinol hydrogen quartet at 212 cps (J = 7 cps) or at 217 cps (J = 7 cps)

(26) A. D. Argoudelis, J. A. Fox, D. J. Mason, and T. E. Eble, *J. Am. Chem. Soc.*, **86**, 5044 (1964); also presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, N. Y., Oct 26–28, 1964.

(27) K. W. Bartz and N. F. Chamberlain, *Anal. Chem.*, **36**, 2151 (1964).

Table IV. Characteristic Absorptions of S-Alkyl Analogs of Lincomycin Hydrochloride[a]

S substituent	Frequency, cps (*J*, cps) S-C*H*	C-C*H*$_3$	Anomeric H
Methyl	128	...	322 (5.5)
Ethyl	158 (7.5)	75	327 (5.5)
Isopropyl	178 (7.0)	78	331 (5.5)

[a] D_2O was the solvent.

for *cis* or *trans* configuration, and a methyl triplet at 68 and 72 cps, respectively.

When the R_3 substituent was an alkyl group other than methyl, the spectrum showed minor changes. The absence of the S-methyl singlet and appearance of the appropriate S-alkyl absorptions were noted, together with a small shift in the absorption frequency of the anomeric hydrogen (see Table IV).

When the 7-hydroxyl was replaced by a chlorine atom XV, profound changes were observed in the carbinol region of the spectrum (see Table II). It is important to note that the intramolecular hydrogen bond is also destroyed and the $J_{5,6}$ and $J_{6,7}$ coupling constants indicate a side-chain conformation similar to VIa.

Experimental Section

Nmr spectra were observed on a Varian A-60 spectrometer using solutions (*ca.* 0.4 ml, 0.3 *M*) of the samples in chloroform-*d* or deuterium oxide. Spectra were calibrated with internal tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (SDSS).[9] Spectra are calibrated in cps at 60 Mc to allow discussion of portions of multiplets.[28] Spin-decoupling experiments were performed with a Varian HR-100 spectrometer using a V-3521 integrator for field-sweep decoupling.[12]

The preparation of degradation products, derivatives, and analogs used in this study is described elsewhere.[3,5-7,21,22,26]

(28) G. Slomp, *J. Am. Chem. Soc.*, **84**, 673 (1962).

Lincomycin. V. Amino Acid Fragment[1]

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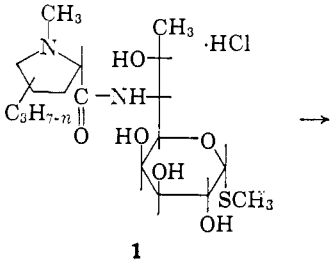
Contribution from the Research Laboratories of the Upjohn Company, Kalamazoo, Michigan. *Received December 20, 1966*

Abstract: The amino acid derived from the cleavage of the antibiotic lincomycin is shown to be *trans*-1-methyl-4-*n*-propyl-L-proline. A partial synthesis of lincomycin is described.

Cleavage of the antibiotic lincomycin hydrochloride (**1**) into methyl thiolincosaminide (**2**) and 1-methyl-*n*-propylproline (**3**) was described in previous papers of this series.[2,3] The determination of the position of the *n*-propyl substituent and the configuration at carbon atoms C_2 and C_4 in the proline fragment is now reported.



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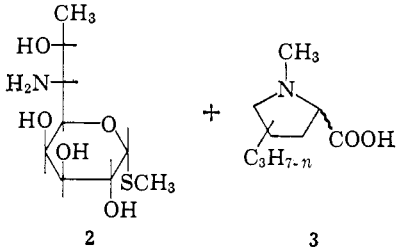

2 **3**

The over-all plan was to establish the position of the alkyl group and confirm the absolute stereochemistry of C-2 by synthesis of 3-, 4-, and 5-*n*-propyl-1-methyl-L-proline[4a] and then to determine the absolute configuration of C_4 by degradation.

The synthesis of 1-carbobenzoxy-4-methylene-L-proline (**5**) resulting from the action of methylenetriphenylphosphorane on 1-carbobenzoxy-4-keto-L-proline (**4**) was recently described.[4b] Under similar conditions *n*-propylidenetriphenylphosphorane failed to give the desired 4-propylidene compound **6**. However, a modification of the recently described sodium methylsulfinylcarbanion–dimethyl sulfoxide procedure[5] afforded **6** in 55% yield.

Although the double bond of **6** could be hydrogenated over a platinum catalyst to give **7**, concomitant hydrogenation and hydrogenolysis of **6** to yield **8** was generally more convenient. Reductive methylation of **8** afforded the methylated amino acid **9** in high yield. The saturated amino acids **7**, **8**, and **9** were obtained as *cis*–*trans* isomers, but separation of these isomers could not be achieved through chromatography, electrophoresis, or fractional crystallization.

(1) Presented at the 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1964; Abstracts of Papers, p 6P. For a preliminary report *cf.* H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. Schroeder, G. Slomp, and R. R. Herr, *J. Am. Chem. Soc.*, **86**, 4223 (1964).

(2) R. R. Herr and G. Slomp, *J. Am. Chem. Soc.*, **89**, 2444 (1967).

(3) W. P. Schroeder, B. Bannister, and H. Hoeksema, *ibid.*, **89**, 2448 (1967).

(4) (a) Rotational data suggested the L configuration for the carboxyl group. See ref 2. (b) M. Bethell, G. W. Kenner, and R. C. Sheppard, *Nature*, **194**, 864 (1962). The authors are indebted to Professor G. W. Kenner for experimental details of this reaction prior to publication.

(5) R. Greenwald, M. Chaykovsky, and E. J. Corey, *J. Org. Chem.*, **28**, 1128 (1963).