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STRUCTURES OF LL-AC541 AND LL-AB664

NEW STREPTOTHRICIN-TYPE ANTIBIOTICS

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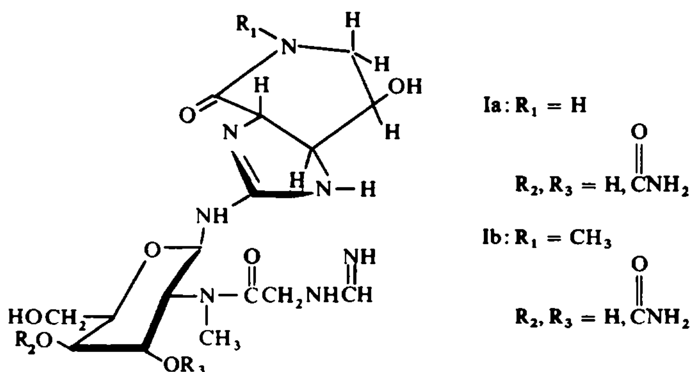
Abstract—Structures for LL-AC541, LL-AB664, and two other related antibiotics are proposed. These compounds appear to be the first reported streptothricin-type antibiotics which do not contain β-lysine.

ANTIBIOTICS LL-AC541 and LL-AB664 are similar, very basic, water-soluble compounds produced by streptomycetes.[1–3] The following studies have resulted in the proposal of structures Ia and Ib for LL-AC541 and LL-AB664, respectively.



LL-AC541 was isolated by carbon and ion-exchange chromatography as an amorphous hydrochloride salt (C, 37·61; H, 6·12; N, 19·63; O, 24·40; Cl, 12·79); $[\alpha]_D^{25}$ −58° (*c*, 1·09, water), m.p. 200–215° d, no absorption from 220 mμ to 400 mμ[2]. Hydrolysis of the antibiotic with 3 N HCl at reflux for 5 hr yielded glycine, ammonia, carbon dioxide, formic acid, N-*guan*-streptolidyl N′-methyl-β-D-gulosaminide (II) and small amounts of streptolidine (III), N-methyl-α-D-gulosamine (IV), and formiminoglycine (V).

II was isolated from the hydrolysate as an amorphous hydrochloride salt following cellulose and charcoal chromatography. It gave positive ninhydrin and Weber tests and a negative Sakaguchi reaction. Treatment with 3 N HCl at 100° for 5 hr yielded

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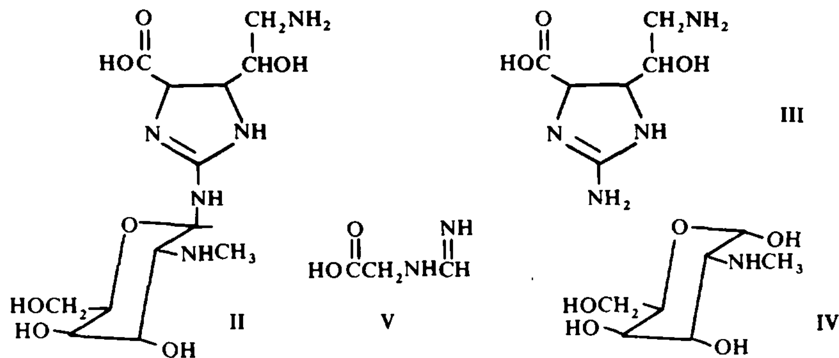
mainly unreacted starting material and small amounts of streptolidine and an amino sugar. Hydrolysis with 6 N HCl at 120° (sealed vial) caused considerable charring and streptolidine and methylamine were the only identifiable products.



IV was recovered from the antibiotic hydrolysate by chromatography as a crystalline hydrochloride salt $C_7H_{15}NO_5 \cdot HCl$. Evidence for a methylamino group was derived from the NMR spectra of the sugar, II, and the antibiotic all of which had 3-proton singlets attributed to the N-Me group. In addition, methylamine was detected in 6 N acid hydrolysates of II and the antibiotic. Evidence that IV is a 2-deoxy-2-methylamino sugar was obtained from a 100 MHz spin-decoupling study of II. The anomeric proton (δ 5·77, J = 9·7) of II was coupled to a single proton at C_2 absorbing at δ 4·04 whose chemical shift was consistent for a methine proton on carbon bonded to the methylamino group. There were peaks at δ 3·71 and δ3·34 corresponding to the streptolidine aminomethylene and methylamino groups, respectively. Immediately downfield from the C_2 proton was the doublet (δ 4·26) of the C_6 hydroxymethyl group of the sugar and below this were the other methine protons of the sugar and the streptolidine ring. The corresponding compound, N-*guan*-streptolidyl β-D gulosaminide (VI)[5] from streptothricin gave, except for an N-methyl absorption, a very similar NMR spectrum with practically the same chemical shifts and coupling constants for the C_1 and C_2 protons of the sugar.

The order of magnitude of the coupling constants (Table 1) indicated three possibilities for the ring protons of the sugar in the chair conformation (Table 2), each of which could exist as the D- or L-isomer. These possibilities correspond to the gulosamine, mannosamine and talosamine configurations. A consideration of mutarotation values[6] (Table 2) has allowed a provisional choice of the D-gulosamine configuration for IV. This assignment assumes that the N-Me substituent has little effect on the rotation which is substantiated in the case of α-D-glucosamine hydrochloride ($[\alpha]_D$ +100° initial, +72° final)[6] and N-methyl-α-L-glucosamine hydrochloride ($[\alpha]_D$ −103° initial, −88° final).[7] Hydrochloride salts of methyl 2-amino-2-deoxy-4,6-di-O-methyl-β, D-glucopyranoside ($[\alpha]_D$ −2°) and its N-Me derivative ($[\alpha]_D$ −1°) represent another related model system.[8] Although caution is obviously necessary when defining stereochemistry by such types of optical rotations, we believe the differences observed in this case are sufficient to allow assignment.

Chemical tests and other physical properties of IV are consistent with the proposed structure and are very similar to those of N-methyl-L-glucosamine. Both sugars gave positive Tollens and Elson–Morgan tests and essentially no reaction with ninhydrin. Paper chromatography and high voltage paper electrophoresis in a number of systems did not separate these two compounds.

TABLE 1. COUPLING CONSTANTS OF AMINO SUGAR HYDROGENS IN II

Carbon Atom	δ	Couplings Observed (Hz)
1	5·77	$J_{12} = 9·7$
2	4·04	$J_{23} = 3·1$
3	4·88	$J_{34} = 4·0$
4	4·41	$J_{45} = < 1·0$
5	4·72	$J_{56} = 6·0$
6	4·26	

TABLE 2. POSSIBLE CONFIGURATIONS OF THE SUGAR IN II

Configuration[a] in II	Corresponding Amino Sugar	Mutarotation Values of D-Isomer Amino Sugar Hydrochlorides	
		Initial	Final
$C_{1a}C_{2a}C_{3e}C_{4e}C_{5a}$	D- or L-gulosamine	+40°	−19°
$C_{1a}C_{2a}C_{3e}C_{4a}C_{5e}$	D- or L-mannosamine	+3°	+3°
$C_{1a}C_{2a}C_{3e}C_{4e}C_{5e}$	D- or L-talosamine	+3°	+6°
	LL-AC541 sugar (IV)	+39°	−22°

[a] Denotes carbon atom of the hexose and an axial (a) or equatorial (e) configuration of the corresponding proton.

The negative Elson–Morgan tests for II indicate that the sugar was linked through a glycosidic bond. The large coupling constant of the anomeric proton in II resulting from an axial–axial relationship of C_1 and C_2 protons indicated a pyranose form for N-methyl-D-gulosamine with a β-glycosidic linkage to the streptolidine moiety.[9] The pyranose form was preferred over the furanose since the chemical shifts and coupling constants of the sugar protons of II were almost the same as those of the pyranose ring protons of the very closely related model compound N-*guan*-streptolidyl gulosaminide (VI).* Chemical shift differences of some anomeric protons of pyranose and furanose forms of sugars in deuterium oxide have been reported by Lemieux.[9d]

The unusual acid stability of the glycosidic bond in II is similar to that observed for N-*guan*-streptolidyl gulosaminide (VI) and can be attributed to stabilization from linkage to the 2-amino-imidazolium unit and proximity to a positively charged methylamino group.[5] At this time the assigned attachment of this glycosidic bond to the exocyclic nitrogen of the 2-amino-imidazoline moiety is based on analogy to the

* The chemical shifts for the C_1 and C_6 pyranose protons of VI are given in Table 3. The associated coupling constants are $J_{1,2} = 9·7$ Hz and $J_{5,6} = 5·7$ Hz. Another readily distinguishable signal of the sugar moiety was the C_2 proton at δ 4·21 ($J_{2,3} = 3·2$ Hz).

TABLE 3. SUMMARY OF NMR SPECTRA OF ANTIBIOTICS AND THEIR DEGRADATION PRODUCTS[a]

Protons	Multi-plicity	LL-AC541 (Ia)	Deformimino LL-AC541 (VIIa)	II	LL-AB664[b]	VIII[b]	VI	Formimino glycine (V)	Sugar (IV) from LL-AC541
N—CH_3	s	3·53	3·53	3·34	3·39, 3·55	3·27, 3·30	—	—	3·35
CH_2 (C_6 sugar)	d	4·17	4·18	4·26	4·22	4·25	4·27	—	4·25
CH_2 (glycine)	s	4·92	4·56	—	4·90	—	—	4·79	—
Anomeric	d	6·10	6·08	5·77	6·06	5·77	5·75	—	5·83
Formimino	s	8·40	—	—	8·47	—	—	8·43	—

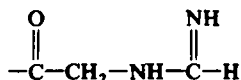
[a] Chemical shifts expressed as δ from TMS used as an external reference. All spectra were obtained in D_2O at 60 MHz. Compounds were hydrochloride salts except for LL-AB664 which was a sulfate.

[b] Chemical shifts were adjusted from internal DDS to external TMS reference by addition of δ 0·47.

structure of the corresponding glycoside from streptothricin. The similarities between the rotations and chromatographic properties of II and N-*guan*-streptolidyl gulosaminide (reported $[\alpha]_D$ $-22.4°$)[5] are consistent also with the proposed structure for II.

Quantitative determinations of the ninhydrin-positive fragments in the antibiotic hydrolysate by means of an amino acid autoanalyzer gave the following relative molar ratios: glycine, 1·0; ammonia, 2·3; streptolidine, 0·2; N-*guan*-streptolidyl N'-methylgulosaminide, 0·7. Assays for formic acid and carbon dioxide liberated in the hydrolysis gave approximately equal moles of each. Additional quantitative data were obtained from the NMR spectrum of the antibiotic which had a 1-proton singlet attributed to a formyl group and a 3-proton singlet for the N-Me group (Table 3). Consideration of all of the analytical data indicated the following molar ratios of primary fragments from the antibiotic: ammonia, 2; carbon dioxide, 1; formic acid, 1; glycine, 1; N-*guan*-streptolidyl N'-methylgulosaminide, 1.

The detection of a small amount of formiminoglycine (V) in the hydrolysate provided evidence for the following structural unit in the antibiotic.



The NMR spectrum of the intact antibiotic like that of the formiminoglycine had a 1-proton singlet for the formimino group (δ 8·40) and a 2-proton singlet (δ 4·92) for the glycyl residue which is in agreement with this structural unit. The lack of absorption bands for a protonated amino acid ester in the IR spectrum[10] of the dihydrochloride salt of the antibiotic, and the hydrolysis characteristics of the antibiotic suggested that the formiminoglycyl residue is joined through an amide rather than ester linkage. A nitrogen available for such a linkage is presumably that of the amino sugar. The signal in the NMR for the N-methyl group of the sugar was only 0·19 ppm lower field in the antibiotic than in II hydrochloride, as might be predicted from the proposed structure.*

The strong basic character of the antibiotic with apparent p*Ka*'s of 6·9 and 10·2 showed that a free carboxyl group was not present.[2] Unlike streptolidine and N-*guan*-streptolidyl N'-methylgulosaminide (II), LL-AC541 does not give a ninhydrin reaction thus showing that the amino group is masked. The masking of both the carboxyl and amino groups of the streptolidine moiety in the antibiotic can be accommodated by a cyclic lactam structure which is substantiated by comparison of NMR spectra. An outstanding feature of the NMR comparisons (Table 4) was the change in the J_{ab} coupling constant from 5·0 Hz for the open-chain form with the dihedral angle of the protons approximately 120° to 14·8 Hz for the lactam form with a dihedral angle near 180°. The approximate bond angles were derived from models. These coupling constants are somewhat higher than predicted from the Karplus relationship which is attributed to the influence of the guanidinium group.[11] As might be expected, the lactam form showed a greater nonequivalence of the methylene protons than the open-chain form.

The NMR results also confirm the relative stereochemistry of the streptolidine ring protons proposed by Carter *et al.*[4] and established the relative configuration

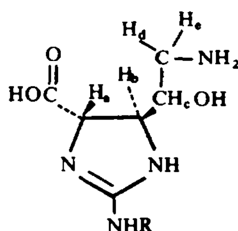
* Under the same conditions the N-Me signals for dimethylamine hydrochloride were at δ 3·23 whereas those for N, N-dimethylacetamide were at δ 3·26 and δ 3·43.

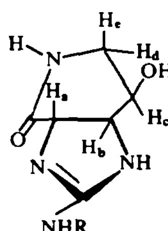
about the remaining asymmetric center. The latter was assigned from the J_{bc} of the streptolidine moiety in LL-AC541 (Table 4). An investigation with molecular models suggested that the lactam ring was in a slightly distorted boat conformation.

TABLE 4. STREPTOLIDINE MOIETY – COMPARISON OF 100 MHZ NMR SPECTRA[a]

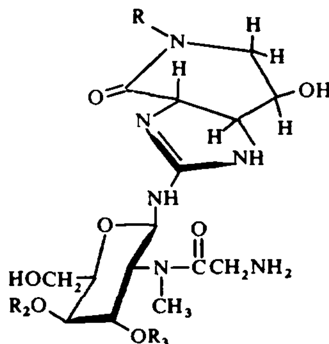
Streptolidine (III)		Streptolidine-sugar (II)		LL-AC541 (Ia)	
δ	Observed couplings	δ	Observed couplings	δ	Observed couplings
H_a 5·07	J_{ab} 4·7	H_a 5·09	J_{ab} 5·0	H_a 5·16	J_{ab} 14·8
H_b 4·74	J_{bc} 3·6	H_b 4·80	J_{bc} 3·5	H_b 4·59	J_{bc} 2·7
H_c 4·59	J_{cd} 3·4	H_c 4·63		H_c ~5·20	J_{cd} 5·5
H_d 3·76	J_{ce} 9·8	$H_{d,e}$ ~3·71[b]		H_d 4·32	J_{ce} 1·2
H_e 3·54	J_{de} 13·2			H_e 3·89	J_{de} 15·0

[a] All chemical shifts are based on an external reference of tetramethylsilane. Number systems for open-chain and lactam forms:

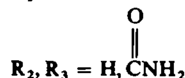




[b] Exact values were not obtained for this portion of the spectrum.

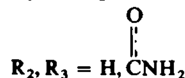


VIIa: R_1 = H



R_2, R_3 = H, $\overset{O}{\overset{\|}{C}}NH_2$

VIIb: R_1 = CH_3



R_2, R_3 = H, $\overset{O}{\overset{\|}{C}}NH_2$

The remaining fragments of the antibiotic, namely one mole of carbon dioxide and one mole of ammonia are presumably present as a carbamate group. This assignment rests on the consistency of the structural features and properties of LL-AC541 with those described with streptolin and streptothricin. The location of this group is most likely on either the C_3 or C_4 OH groups of the sugar. Substitution at the C_6 position should result in a chemical shift* to lower field in the NMR for the doublet of the C_6 methylene in II compared to the intact antibiotic. Such a shift was not observed.

* The $\Delta\delta$ for the methylene group of ethyl carbamate and ethanol was 0·42 when the spectra were obtained in D_2O with an external reference.

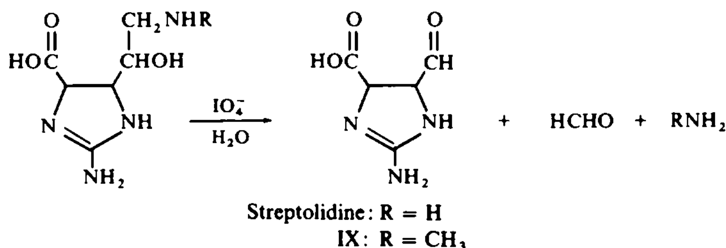
The evidence presented above is consistent with structure Ia for LL-AC541.

Treatment of LL-AC541 dihydrochloride with a solution of ammonium hydroxide-methanol or prolonged standing in methanol resulted in conversion to a new antibiotic (VIIa) which was also observed in the original culture filtrate.[2]

The resulting antibiotic was obtained as an amorphous hydrochloride salt. The NMR spectrum lacked the formimino absorption and the singlet for the glycine moiety appeared more shielded ($\Delta\delta$ 0·4). In all other features, the NMR spectrum of this new antibiotic was essentially the same as that of LL-AC541. It gave a positive ninhydrin reaction and the hydrolysate contained 1 mole less ammonia than that of LL-AC541. These characterization data and the type of conversion reaction involved are consistent with structure VIIa for this new antibiotic which differs from the starting antibiotic in its lack of the N-formimino group. The extreme lability of the formimino group is consonant with previously reported data on formiminoglycine,[12] and partial degradation during titration would explain the anomalous p*Ka* (6·9) and the large neutralization equivalent (426) of LL-AC541.[2]

LL-AB664 structure proposal (Ib) is the same as for LL-AC541 (Ia) with an additional methyl group substituted on the nitrogen of the lactam ring. Evidence for this structure is based primarily on the characterization of hydrolysis products. Hydrolysis of LL-AB664 with 3 N HCl produced ammonia and glycine in a 2:1 molar ratio as the only ninhydrin-positive fragments. A small amount of formiminoglycine and a high yield of a streptolidine amino sugar (VIII) were also obtained. More vigorous hydrolytic conditions yielded ammonia and glycine in a 2:1 molar ratio, methylamine and a streptolidine derivative (IX).

Unlike streptolidine from streptothricin and the LL-AC541 antibiotic, IX gave a very weak reaction with ninhydrin. A comparative study of streptolidine and IX indicated that both produce the same aldehyde on periodate cleavage, but IX produced methylamine instead of ammonia.



Streptolidine: R = H
IX: R = CH_3

The NMR spectrum of VIII (Table 3) was the same as that of II except for an additional N-methyl signal attributed to the group attached to the streptolidine moiety. Spin-decoupling studies of VIII gave essentially the same results as II and suggested that LL-AB664 contained the same sugar as LL-AC541. Further hydrolysis of VIII produced a Tollens positive fragment with the same R_f values by paper chromatography as the N-methylgulosamine. The properties of the antibiotic and its hydrolysis products are consistent with structure Ib for the antibiotic. Therefore, LL-AB664 is an N-methyl derivative of LL-AC541.

Treatment of LL-AB664 with methanolic ammonia resulted in conversion to a closely related antibiotic VIIb which presumably resulted from the loss of the formimino group as noted for the LL-AC541 antibiotic.

LL-AC541 and LL-AB664 and their closely related deformimino derivatives appear to be the first reported streptothricin-type antibiotics that do not contain β-lysine; also LL-AC541 and LL-AB664 are apparently the first antibiotics reported to have a formiminoglycyl grouping.

Soon after the initial publication on the LL-AC541 and LL-AB664 antibiotics,[1,3] several similar or identical antibiotics were reported by groups in Japan. Antibiotic SF-701 was reported to be a streptothricin-type probably containing N-methylglycine instead of β-lysine.[13] Antibiotics E-749-C[14] and BY-81[15] appeared to be the same as LL-AC541 and antibiotic BD-12[15] and sclerothricin[16], the same as LL-AB664.

EXPERIMENTAL

NMR spectra were determined at 60 MHz in D_2O with TMS as an external reference unless stated otherwise. Spin-decoupling was accomplished by the frequency-sweep method with a 100 MHz Varian spectrometer. UV spectra were determined in absolute MeOH. M.ps are uncorrected.

Amino acid autoanalyzer studies. Pure samples (~2 mg) of the LL-AC541 dihydrochloride[2] and LL-AB664 sulfate[3] were partially hydrolyzed (sealed vials) with 3 N HCl at 100–110° for 5 hr or totally hydrolyzed with 6 N HCl at 120–130° for 16 hr. The residues from the hydrolysates were then studied with the Technicon amino-acid autoanalyzer by eluting the analyzer column continuously with a pH 5·0 buffer.[17] Retention times and color yields of the individual components were determined with pure samples obtained from preparative hydrolyses described below.

Partial hydrolysis of LL-AC541 *on a preparative scale.* The hydrolysis was conducted on a sample of LL-AC541 which contained approximately 50% antibiotic with the impurity being essentially NaCl. The partially purified LL-AC541 (6·0 g) was dissolved in 150 ml of 3 N HCl and heated under reflux for 5 hr. A stream of purified N_2 was passed through the refluxing soln and then into 350 ml of 2 N NaOH to collect CO_2 evolved. The hydrolysate soln was then evaporated *in vacuo* to a residue that was subjected to chromatography and a distillate which was assayed for formic acid.

The CO_2 was determined as $BaCO_3$ by essentially the same procedure described by Carter *et al.*[18] The carbonate weighed 1·02 g corresponding to 5·2 mmoles CO_2.

Aliquots of the distillate were assayed for formic acid by reducing the acid to formaldehyde which was determined with chromotropic acid.[19] The total distillate contained 3·9 mmoles formic acid.

The residue from the hydrolysis was chromatographed on a dry-packed column (4 × 75 cm) of Whatman cellulose powder, standard grade, prewashed with elution solvent t-BuOH, AcOH, water (4:1:1). To charge the column, the residue was dissolved in water and adsorbed onto 9 g cellulose powder which was then dried and packed onto the top of the column. Fractions were assayed by spot tests and paper chromatography. The flow rate of the column was 30 ml/hr. Results of the separation are summarized in Table 5.

TABLE 5. COLUMN FRACTIONS FROM THE PARTIAL HYDROLYSATE OF LL-AC541

Fraction	Ml of column effluent	Components	Weight
A	600–2,100	Glycine, amino sugar, formiminoglycine, inorganic salts	3·17 g
B	3,000–5,100	Streptolidine	0·27 g
C	6,000–11,700	N-*guan*-streptolidyl N′-methylgulosaminide	1·64 g

Fraction A, 1·3 g was dissolved in a minimum amount water and introduced onto an 18 × 350 mm column of a polystyrene sulfonic acid-type ion-exchange resin (H^+, 200–400 mesh). The column was developed with 0·3 N HCl and fractions which contained approximately 7·5 ml were collected every 15 min. Tollens,[20] ninhydrin and hypochlorite-iodide[21] spot tests indicated that fractions 63–70 contained the sugar, fractions

100–125 the glycine and fractions 150–198 the formiminoglycine. The combined yields from this and a similar run with 1·5 g of fraction A gave 45 mg of crude sugar, 159 mg of crude formiminoglycine and 341 mg (4·5 mmoles) of glycine.

Partial hydrolysis of LL-AB664 *on a preparative scale.* LL-AB664 sulfate (1·46 g) was hydrolyzed and the hydrolysate chromatographed on cellulose and on a strong acid ion-exchange resin by a very similar procedure as used for LL-AC541. The cellulose column, 6 × 60 cm, was eluted with approximately 2,000 ml of t-BuOH, AcOH, water (4:1:1) and then 2,000 ml of t-BuOH, AcOH, water (2:1:1). Products were detected by paper chromatography and spot tests.

The results from the cellulose column are summarized in Table 6.

TABLE 6. COLUMN FRACTIONS FROM THE PARTIAL HYDROLYSATE OF LL-AB664

Fraction	Ml of column Effluent	Components	Weight
A	1,250–1,400	Crude formiminoglycine	175 mg
B	1,600–2,000	Glycine and inorganic salts	125 mg
C	3,000–4,000	Crude N-*guan*-N′-methylstreptolidyl N″-methylgulosaminide	865 mg

Streptolidine (III). When fraction B from the partition column of the LL-AC541 hydrolysate was dissolved in 0·5 N HCl and evaporated, the streptolidine crystallized as a dihydrochloride. The crystalline product was washed with MeOH-acetone (2:1) to remove some dark-colored imputities. The resulting crystalline product had $[\alpha]_D^{25}$ +55·3 ±2·9° (*c*, 1·01, water), m.p. 215° d. It gave positive ninhydrin and Weber, but negative Elson–Morgan and Sakaguchi tests. The UV spectrum showed only end absorption and the IR and NMR spectra were identical with those reported for streptolidine dihydrochloride,[22,23] (Found: C, 26·96, 27·23; H, 5·39, 5·33; N, 21·33; Cl, 27·29. Calc. for $C_6H_{12}N_4O_3 \cdot 2\,HCl$: C, 27·60; H, 5·41; N, 21·46; Cl, 27·16%).

Further confirmation of identity was obtained by direct chromatographic and spectral comparisons to streptolidine dihydrochloride obtained from streptothricin by the same procedure.[4]

N-*Methyl-α-*D*-gulosamine* (IV). The sugar fraction from the ion-exchange column of the LL-AC541 hydrolysate was dissolved in water and passed through a column (6 × 30 mm) of charcoal, diatomaceous earth (3:2 by volume) to remove colored impurities. The residue, after evaporation of the eluate, was readily crystallized from EtOH-water to give the colorless hydrochloride salt, m.p. 155° d, $[\alpha]_D^{25}$ +39° initial (extrapolated), −22° final (*c*, 0·785, water). It gave only a very slight ninhydrin, but clearly positive Elson–Morgan and Tollens reactions· The compound could not be separated from N-methyl-L-glucosamine* by paper electrophoresis at pH's 2·0, 3·8, 6·5 and 10·0 nor by paper chromatography with the systems pyridine, AcOH, water (10:7:3); EtOAc pyridine, water (12:5:4); 1-BuOH, pyridine, water (6:4:3) and 1-BuOH, AcOH, water (4:1:5); γ_{max}^{KBr}: 3350, 2950, 1550, 1460, 1420, 1360, 1315, 1235, 1185, 1166, 1130, 1097, 1067, 1036, 1025, 972, 963, 916, 883, 875, 825, 763 cm^{-1}; NMR (after mutarotation) δ 3·35 (s, $N\text{-}CH_3$), δ 4·25 (apparent d, $-CH_2OH$), and δ 5·83 (d, anomeric proton). (Found: C, 36·48; H, 6·89; N, 6·10. $C_7H_{15}NO_5 \cdot HCl$ requires: C, 36·60; H, 7·02; N, 6·10%).

N-*Guan-streptolidyl* N′-*methyl-β-*D*-gulosaminide* (II). II was eluted from the partition column as a single broad-tailing band determined on a dry weight basis and collected in 4 fractions. The optical rotations of the solids from these 4 fractions varied significantly. When each was passed through a small column of charcoal with water, the eluates yielded colorless amorphous solids which appeared to be equivalent and homogeneous by optical rotations and autoanalyzer chromatography.[17] Attempts to crystallize this product were unsuccessful. It had only end absorption in the UV region, m.p. 175–185° d, $[\alpha]_D^{25}$ −27 ±4° (*c* 0·813, water), γ_{max}^{KBr}: 3300 b, 1655, 1605, 1490 w, 1450 w, 1390, 1287, 1215 sh, 1155 sh, 1057 b, 952 w, 920 w. Ninhydrin and Weber tests were positive, Elson–Morgan and Sakaguchi reactions were negative. (Found: C, 30·90; H, 6·39; N, 13·72; Cl, 18·45. $C_{13}H_{25}N_5O_7$: 2·7 $HCl \cdot 2{\cdot}5\, H_2O$ requires: C, 30·80; H, 6·50; N, 13·82; Cl, 18·89%).

* Isolated as the hydrochloride salt from a hydrolysate of streptomycin.

Hydrolyses of II with 3 N and 6 N HCl were conducted on a milligram scale as previously described and the products were identified by paper chromatography. The partial hydrolysis with 3 N HCl yielded mainly starting material and small amounts of streptolidine and the amino sugar, whereas the total hydrolysis gave only streptolidine and methylamine as identifiable products.

Glycine and ammonia. These fragments were identified in the antibiotic hydrolysates by amino acid autoanalyzer retention times. In addition, the isolated crystalline glycine hydrochloride was identified by IR spectrum and paper chromatography.

Formiminoglycine (V). Recrystallization with MeOH-acetone of the crude formiminoglycine from the ion-exchange chromatography of LL-AB664 hydrolysate gave formiminoglycine hydrochloride as colorless crystals. NMR δ 4·32 (s, CH_2), δ 7·92 (s, —C—H). (Found: C, 26·00; H, 5·10; N, 20·22; Cl, 25·59. Calc. for $C_3H_6N_2O_2 \cdot HCl$: C, 26·45; H, 5·18; N, 20·30; Cl, 25·50%). Direct comparisons with synthetic formiminoglycine hydrochloride[12] showed the compounds to be identical. Formiminoglycine was identified by paper chromatography and paper electrophoresis in fractions from the ion-exchange chromatography of LL-AC541 hydrolysate.

N-*Guan-streptolidyl gulosaminide* (VI). Hydrolysis of streptothricin produced by a *Streptomyces* sp. (Lederle. L-1689) and chromatography of the hydrolysate by essentially the same procedure as used for the LL-AC541 antibiotic yielded N-*guan*-streptolidyl gulosaminide which had approximately the same retention time on the cellulose column as II. Further purification by passing an aqueous solution through charcoal provided a colorless, amorphous hydrochloride salt. The NMR spectrum of this compound was extremely similar to that of II except that it lacked the N-Me signal. When the compound was hydrolyzed with 3 N HCl for 5 hr, it remained largely unreacted but small amounts of streptolidine and D-gulosamine were detected by paper chromatography. Milligram quantities of the latter compounds were obtained from the hydrolysate of the antibiotic itself by the procedures used to purify the corresponding fragments from LL-AC541. Streptolidine was obtained as the crystalline dihydrochloride salt and could not be differentiated from III. The sugar was crystallized from EtOH-water to obtain the α-D-gulosamine hydrochloride as colorless crystals, m.p. 148–155° d. $[\alpha]_D^{25}$ +32·8° (5 min) −15·3° final (*c* 0·913, water). (Found: C, 33·40; H, 6·66; N, 6·40. Calc. for $C_6H_{13}NO_5 \cdot HCl$: C, 33·42; H, 6·54; N, 6·49%).

N-*Guan*-(N′-*methylstreptolidyl*) N″-*methyl*-β-D-*gulosaminide* (VIII). Crude compound VIII, obtained from partition chromatography of the hydrolysate of LL-AB664, was dissolved in water and passed through a column of charcoal, diatomaceous earth (1:4 wt). The eluate was freeze-dried to obtain the amorphous hydrochloride salt. $[\alpha]_D^{25}$ −21° ±3° (*c* 1·04, water). The NMR and 100 MHz spin-decoupling spectra of the salt (VIII) were practically the same as those of II from LL-AC541 except that they had an additional N-Me signal. Further hydrolysis of VIII with 3 N HCl yielded N-methyl-streptolidine and a sugar having the same R_f value (0·37) by paper chromatography (t-BuOH, AcOH, water, 2:1:1) as the N-methyl-D-gulosamine from LL-AC541.

N-*Methyl-streptolidine* (IX). LL-AB664 (100 mg) was hydrolyzed in 10 ml of 6 N HCl at 120° for 18 hr. Preparative paper chromatography of the hydrolysate provided a Weber-positive, ninhydrin-negative, streptolidine-like fragment of R_f 0·24 in the system t-BuOH, AcOH, water (2:1:1). This material was eluted from the paper and reacted with periodate. The product of this reaction was shown to contain methylamine both by paper chromatography and paper electrophoresis. In addition, the electrophoresis study demonstrated two Tollens positive fragments that had the same mobilities as the periodate oxidation products of streptolidine obtained in a parallel experiment.

Mild basic degradation of LL-AC541 *and* LL-AB664. LL-AC541 dihydrochloride, 100 mg, was dissolved in a soln of 6·5 ml of methanol plus 3·5 ml of concentrated NH_4OH and allowed to stand at room temp for 30 min. The residue from evaporation of the solvent *in vacuo* was dissolved in 10 ml water and adsorbed onto a weakly acidic cation-exchange column (8 × 50 mm, 100–200 mesh). The column was washed with 20 ml water and eluted with 10 ml 0·3 N HCl. The residue from the acid fraction was an amorphous hydrochloride salt of deformimino LL-AC541 (VIIa). The NMR spectrum of this compound was very similar to that of LL-AC541 except that it lacked the formyl signal and showed the glycine peak shifted upfield (Table 3). An autoanalyzer study of the hydrolysate (prepared with 3 N HCl) gave the following mole ratios: glycine, 1·0; streptolidine, 0·07; ammonia, 1·3; streptolidine-sugar (II), 0·63. Paper chromatography with 90% phenol, *m*-cresol, AcOH, pyridine, water (200:50:8:8:150) differentiated the new antibiotic from LL-AC541 (R_f 0·58) but not from a related antibiotic (R_f 0·40) frequently found in the fermentations for LL-AC541. The antibiotic zones were detected by bioautography against

Klebsiella pneumoniae. A similar treatment of LL-AB664 with MeOH-aqueous ammonia resulted in conversion to another antibiotic which had by chromatography R_f 0·65 while LL-AB664 had R_f 0·80.

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