Tetrahedron Letters No.42, pp. 4187–4192, 1967. Pergamon Press Ltd. Printed in Great Britain.

PARTIAL STRUCTURE OF ANTIBIOTIC LL-AC541

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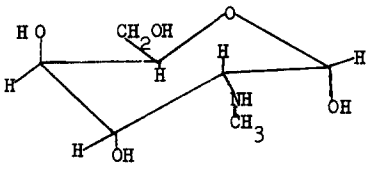
(Received in USA 14 July 1967)

In the antibiotic program conducted in our laboratories, a strain of Streptomyces hygroscopicus isolated from soil was found to produce a water-soluble, strongly basic antibiotic active against gram-negative and gram-positive bacteria. The antibiotic, called LL-AC541, was isolated by carbon and ion-exchange chromatography as an amorphous hydrochloride salt (Found: C, 35.68; H, 6.04; N, 18.58; O, 24.35; Cl, 12.31), $[\alpha]_D^{25}$ -58° (c 1.09, water), mp 200-215° d, no absorption from 220 mμ to 400 mμ.[1]

Hydrolysis of the antibiotic with 3 N hydrochloric acid at reflux temperature for 5 hr yielded glycine, streptolidine, ammonia, carbon dioxide, formic acid, a reducing compound (I), and a very basic compound (II). Streptolidine, isolated as the crystalline dihydrochloride salt, mp~215° d, $[\alpha]_D^{25}$ +55.3 (c 1.01, water), was identified by direct comparison with an authentic sample obtained by hydrolysis of streptothricin.[2]

Compound I was isolated from the hydrolysate by chromatography on cellulose and then on Dowex 50W-X8 (H^+ form) as a crystalline hydrochloride salt, $C_7H_{15}NO_5$.HCl (Found: C, 36.48; H, 6.89; N, 6.10), mp~155° d, $[\alpha]_D^{25}$ +39° initial (extrapolated), -22° final (c 0.785, water). It has been tentatively identified as N-methyl-α-D-gulosamine from the following evidence.



I

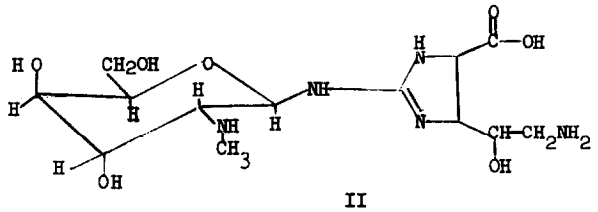
Positive Tollens and Elson-Morgan tests and essentially no reaction with ninhydrin were indicative of an N-substituted 2-amino aldose. The NMR spectra of the hydrochloride salts of I and N-methyl-L-glucosamine* were very similar, both having one N-methyl singlet which was near δ 3.3 and a one-proton doublet near δ 5.5 for the anomeric proton.** Although I and N-methyl-L-glucosamine were not separated by paper chromatography and high voltage paper electrophoresis in a number of systems, their optical rotations and IR spectra showed that they were different isomers. Tentatively, I has been assigned the same configuration as α-D-gulosamine ($[\alpha]_D$ +40° initial, -19° final, or $[\alpha]_D$ +6° at 10 min, -18° final)[3,4] since the mutarotation values of I match more closely those of D-gulosamine than any of the other 2-amino-aldohexoses.[5] This assignment assumes that the N-methyl substituent has little effect on the rotation, which is substantiated in the case of α-D-glucosamine hydrochloride ($[\alpha]_D$ +100° initial, +72° final)[5] and N-methyl-α-L-glucosamine hydrochloride ($[\alpha]_D$ -103° initial, -88° final).[6,7] Further support of this assignment was obtained from spin decoupling studies of compound II, which provided additional evidence for a 2-amino sugar and established the relative configuration at the C_2 and C_3 atoms.

Compound II ($C_{13}H_{25}N_5O_7$) was isolated as an amorphous hydrochloride salt following cellulose and charcoal chromatography of the 3 N hydrochloric acid hydrolysate of the antibiotic (Found: C, 30.90; H, 6.39; N, 13.72; Cl, 18.45) mp 175-185° d, $[\alpha]_D^{25}$ -27° (c 0.813,

* Obtained from streptomycin as a hydrochloride salt, mp 160-161°, $[\alpha]_D^{25}$ -102° initial (extrapolated), -86° final (c 0.969, water).

** Spectra were obtained in deuterium oxide with an external tetramethylsilane reference on a Varian A-60 NMR spectrometer. Spin decoupling studies were performed with a Varian DP60 instrument.

water). II was tentatively identified as N-guan-streptolidyl N-methyl-β-D-gulosaminide on the basis of the following evidence. Ninhydrin and Weber tests were positive, and Elson-Morgan



II

and Sakaguchi tests negative. Following hydrolysis under the conditions used on the antibiotic, streptolidine, a small amount of I, and a considerable amount of unreacted II were detected. Hydrolysis of II with 6 N hydrochloric acid at 120° (sealed vial) caused considerable charring, and streptolidine and methylamine were the only identifiable products in the hydrolysate. The NMR spectrum of the hydrochloride of II had one N-methyl singlet that was near δ3.3, as did I and the intact antibiotic.

The negative Elson-Morgan test for compound II indicated that the sugar is linked through a glycosidic bond. A one-proton doublet at δ 5.80 (J = 10 cps) in the NMR spectrum is consistent for the anomeric proton. The large coupling constant of the anomeric proton resulted from an axial-axial relationship of C_1 and C_2 protons, and suggested the pyranose form for N-methyl-D-gulosamine and a β-glycosidic linkage to the streptolidine moiety.[8] Spin decoupling studies showed that the anomeric proton was coupled to a single proton at δ 4.0, which had four lines due to splitting by the anomeric and C_3 hydrogens. The chemical shift of the C_2 proton was upfield from protons attached to carbon atoms bearing oxygen, and was consistent for a proton on carbon attached to the N-methyl group. The $J_{2,3}$ value (3.5 cps) indicated an axial-equatorial relationship for the C_2 and C_3 protons.

The unusual acid stability of the glycosidic bond in II is similar to that observed for N-guan-streptolidyl gulosaminide isolated from streptothricin, and can be attributed to stabilization from linkage to the 2-amino-imidazoline unit and the proximity to a positively

charged methylamino group.[9] At this time the assigned attachment of this glycosidic bond to the exocyclic nitrogen of the 2-aminoimidazoline moiety is based on analogy to the structure of the corresponding glycoside from streptothricin. The similarities between the optical rotations and chromatographic properties of II and N-guan-streptolidyl gulosaminide (reported $[\alpha]_D$ -22.4°)[9] 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[10] 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[11] and carbon dioxide[12] liberated in the hydrolysis gave approximately a 1/1 molar ratio to each other. The NMR spectrum of the antibiotic had a 1-proton singlet attributed to a formyl group at δ 8.16 and a 3-proton singlet for the N-methyl group at δ 3.18. Consideration of all of the quantitative 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.

Antibiotic LL-AC541 belongs to the streptothricin class since it contains an N-guan-streptolidyl hexosaminide unit, but is distinctive by having an N-methyl amino sugar and glycine, and no β-lysine. This seems to be the first antibiotic of this type which does not contain β-lysine. Most other antibiotics of the streptothricin class appear to differ mainly by the number of β-lysine groups(1-6) per molecule.[13]

Acknowledgement. We thank Mr. L. M. Brancone and staff for microanalyses, Mr. W. Fulmor and staff for spectral data, Mr. M. C. Davies for autoanalyzer determinations, and Dr. J. E. Lancaster and staff for spin decoupling studies.

References

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