

A ^{13}C -NMR SPECTROSCOPIC STUDY OF
 α - AND β -STREPTOMYCIN

K. BOCK and C. PEDERSEN

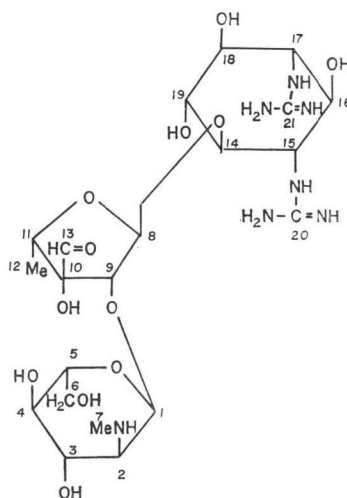
Department of Organic Chemistry, Technical
University of Denmark, DK-2800
Lyngby, Denmark

H. HEDING

Department of Applied Biochemistry, Technical
University of Denmark, DK-2800
Lyngby, Denmark

(Received for publication October 4, 1973)

The structure of streptomycin is well established from chemical,¹⁾ spectroscopic,^{2,3)} and X-ray investigations.⁴⁾ However, the nature of the aldehydic carbon in the streptose moiety remains unknown. Proton NMR

Table 1. ^{13}C -NMR spectra of streptomycin(SM) compound, solvent.

Chemical shifts ^a coupling constants ^b	α -SM sulfate D_2O	α -SM sulfate, D_2O 0.01N NaOH	α -SM sulfate, D_2O 0.01N HCl	α -SM ¹¹ CaCl_2 3HCl D_2O	β -SM 3HCl^c D_2O , 0.01N HCl	Dihydro- SM sulfate D_2O	Methyl streptobio- saminide D_2O
C1	95.5	95.6	95.4	95.3	95.5	94.5	95.7
C2	61.6 ^d	61.6 ^d	61.6 ^d	61.4 ^d	61.4	61.4 ^d	61.3 ^d
C3	70.8 ^e	70.6 ^e	70.6 ^e	70.4 ^e	69.9 ^e	70.3	70.2
C4	70.6 ^e	70.4 ^e	70.3 ^e	70.1 ^e	69.2 ^e	70.3	70.2
C5	72.7 ^f	72.6 ^f	72.6 ^f	72.3 ^f	70.3	72.5 ^e	73.5
C6	62.6 ^d	62.6 ^d	62.6 ^d	62.3 ^d	61.4	62.0 ^d	62.5 ^d
C7	33.5	33.5	33.4	33.3	32.0	33.0	33.4
C8	106.9	106.8	106.8	106.4	107.6	106.7	100.2
C9	78.5 ^g	78.5 ^g	78.5 ^g	78.2 ^g	76.9 ^f	78.7	77.6
C10	83.3	83.3	83.3	82.9	81.7	81.7	82.4
C11	85.5	85.5	85.5	85.5	86.2	84.9	85.8
C12	13.8	13.8	13.8	13.5	12.0	13.8	13.7
C13	90.5	90.6	90.6	90.3	96.1	64.3	90.3
C14	79.1 ^g	79.0 ^g	79.0 ^g	78.7 ^g	77.9 ^f	78.7	
C15	59.6 ^h	59.3 ^h	59.3 ^h	59.1 ^h	57.5 ^g	59.1 ^f	
C16	74.4 ⁱ	74.4 ⁱ	74.4 ⁱ	74.1 ⁱ	73.6	74.3 ^h	
C17	60.0 ^h	60.0 ^h	60.0 ^h	59.6 ^h	58.5 ^g	59.8 ^f	
C18	74.0 ⁱ	73.9 ⁱ	73.9 ⁱ	73.7 ⁱ	73.6	73.7 ^h	
C19	71.8 ^f	71.7 ^f	71.8 ^f	71.7 ^f	81.1	71.5 ^e	
C20	159.1 ^k	159.0 ^k	159.1 ^k	158.6 ^k	157.7	158.8 ^g	
C21	158.5 ^k	158.5 ^k	158.5 ^k	158.2 ^k	157.7	158.3 ^g	
$^1\text{J}_{13\text{CH}_1}$	170					170	170.5
$^1\text{J}_{13\text{CH}_{13}}$	162						162
$^1\text{J}_{13\text{CH}_8}$	174						170

a) Measured at 22.63 MHz with a Bruker WH-90 instrument in 25% solutions relative to 10% internal dioxane=67.4 ppm. b) ± 0.7 Hz using a pulsed decoupling technique. c) Chemical shifts relative to internal methanol=49.3 ppm. d, e, f, g, h, i, k: assignments may be reversed.

shows that a -CHO group is not present.⁵⁾ Some authors have suggested that the aldehyde group forms a "carbinolamine" with the *N*-methyl group^{5,6)} or with a guanidino group.³⁾ The formation of a hemiacetal with one of the hydroxy groups in the glucosamine or streptidine rings was considered less likely for steric reasons.⁵⁾ A hydrated aldehyde group was also excluded for the same reasons.⁵⁾ Actually two tautomeric forms of streptomycin salts are known, namely an amorphous and a crystalline form (α - and β -streptomycin, respectively).⁶⁾

A proton decoupled ¹³C-NMR spectrum of α -streptomycin showed separate signals for all 21 carbon atoms. The assignment (Table 1) was based on a comparison with monosaccharides and cyclitols.⁷⁾ The signals of the aldehydic carbon (No. 13) was assigned by comparison with dihydrostreptomycin. The spectrum of the methyl glycoside of streptobiosamine was identical with that of α -streptomycin except for the 8-carbon atoms of streptidine. The spectrum of α -streptomycin was unaffected by changes in pH. The spectrum of the crystalline calcium chloride complex was identical with that of α -streptomycin.

Uncoupled spectra gave some of the ¹J(¹³CH) values (Table 1). The ¹³CH coupling constant confirms the α -glycosidic nature of the glucosamine part of the molecule.^{8,9)}

The chemical shift (90.5 ppm) of the aldehydic carbon atom indicates that it is on the hydrate form. This is seen by comparison with the spectrum of hydrated acetaldehyde in which the aldehydic carbon is found at 88.9 ppm (¹J_{CH}=162 Hz). A hemiacetal form would probably place C13 close to the anomeric carbons of free sugars (*ca.* 95 ppm).⁷⁾ In a "carbinolamine" C13 would be expected to resonate at higher field. ARONSON *et al.*⁵⁾ suggested that the "carbinolamine" was analogous to the pseudobase of cotarnine. We have measured a ¹³C spectrum of the pseudobase of the closely related alkaloid hydrastinine and found the "carbinolamine-carbon" at 85 ppm.

A spectrum of crystalline β -streptomycin trihydrochloride showed that the aldehydic carbon is shifted downfield to 96.1 ppm. Another carbon atom, either C3 or C4 of the

glucosamine or C19 of the streptidine part, is also shifted downfield. If this is compared with the ¹³C-spectra of disaccharides, in which the two carbon atoms attached to the glycosidic oxygen are shifted downfield,¹⁰⁾ it may be concluded that the aldehydic carbon of β -streptomycin forms a hemiacetal with the hydroxy group of carbon Nos. 3, 4, or 19. The chemical shifts do not allow a distinction between the latter 3 atoms, but DREIDING molecular models indicate that an 8-membered hemiacetal ring with carbon No. 19 of the streptidine ring is most likely. Alternatively, a dimeric hemiacetal may be possible.

Thus we conclude that α -streptomycin contains a hydrated aldehyde group and that β -streptomycin is a hemiacetal.

References

- 1) DYER, J. R.; W. E. MCGONIGAL & K. C. RICE: Streptomycin. II. Streptose. *J. Am. Chem. Soc.* 87: 654~655, 1965
- 2) MCGILVERAY, I. J. & K. L. RINEHART, Jr.: The anomeric linkage of streptose in streptomycin and bluensomycin. *J. Am. Chem. Soc.* 87: 4003~4004, 1965
- 3) CLAES, P.; H. VANDERHAEGHE, J. TOTTÉ & G. SLLINCKX: NMR Spectra of dihydrostreptomycin and some of its degradation products. *Bull. Soc. Chim Belges* 78: 151~158, 1969
- 4) NEIDLE, S., D. ROGERS & M. B. HURSTHOUSE: The crystal and molecular structure of streptomycin oxime selenate. *Tetrahedron Letters* 1968: 4725~4728, 1968
- 5) ARONSON, J.; W. L. MEYER & T. D. BROCK: A molecular model for chemical and biological differences between streptomycin and dihydrostreptomycin. *Nature* 202: 555~557, 1964
- 6) YOUNG, D. P.: Tautomeric forms of streptomycin. *J. Chem. Soc.* 1961: 1337~1342, 1961
- 7) STOTHERS, J. B.: Carbon-13 NMR spectroscopy. p. 458. Academic Press, 1972
- 8) BOCK, K.; I. LUNDT & C. PEDERSEN: Assignment of anomeric structure to carbohydrates through ¹³CH coupling constants. *Tetrahedron Letters* 1973: 1037~1040, 1973
- 9) BOCK, K. & C. PEDERSEN: to be published.
- 10) DORMAN, D. E. & J. D. ROBERTS: Nuclear magnetic resonance spectroscopy. Carbon-13 spectra of some common oligosaccharides. *J. Am. Chem. Soc.* 93: 4463~4472, 1971
- 11) PECK, R. L.; N. G. BRINK, F. A. KUEHL, Jr., E. H. FLYNN, A. WALTI & K. FOLKERS: Streptomyces antibiotics. II. Crystalline streptomycin trihydrochloride-calcium chloride double salt. *J. Am. Chem. Soc.* 67: 1866~1867, 1945