275. Structure of Hygromycin B, an Antibiotic from *Streptomyces hygroscopicus*[1]); The Use of CMR. Spectra in Structure Determination, I

by **N. Neuss*, K. F. Koch*, B. B. Molloy*, W. Day*, L. L. Huckstep*, D. E. Dorman**[2]),** and **J. D. Roberts****

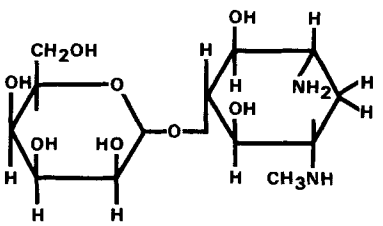
(19 X 70)

*The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, USA
**Gates and Crellin Laboratories of Chemistry (Contribution No. 4148) California Institute of Technology, Pasadena, California, USA[3])

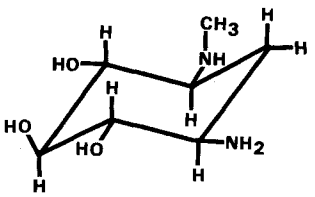
Zusammenfassung. Das Antibioticum Hygromycin B, eine Verbindung $C_{20}H_{37}N_3O_{13}$ aus *Streptomyces hygroscopicus*, wurde hydrolytisch gespalten und die Spaltprodukte charakterisiert und identifiziert. Die Kenntnis ihrer Struktur und die Interpretation von ^{13}C-NMR.- (CMR.)-Spektren führten zur Strukturaufklärung des Antibiotikums.

The isolation and some of the physical and chemical properties of hygromycin B, an antibiotic from *Streptomyces hygroscopicus*, have been first reported in 1958 by *Mann & Bromer* [1].

In 1962 *Wiley et al.* [2] have shown that mild acid hydrolysis of the antibiotic gave rise to two products designated hygromycin B_2 (I), a $C_{13}H_{26}N_2O_8$ compound, and hyosamine (II), a dextrorotatory deoxystreptamine derivative,



Hygromycin B_2 (I)



N-Methyl-2-Deoxystreptamine (II)

$C_7H_{16}N_2O_3$. Both compounds were devoid of antibacterial properties. Hydrolysis under more vigorous conditions, using boiling 0.5 N sulfuric acid, led to the isolation of D-talose (III) as well as hyosamine (II). Structures I and II were assigned on the basis of physical and chemical data. However, due to difficulties in purification of hygromycin B and inconsistent elemental analyses, no empirical formula could be assigned to the antibiotic [2]. Through repeated chromatography of impure hygromycin B on Bio-Rad resin, AG1X2 (200–400 mesh) OH cycle, with water as eluent,

[1]) Presented in part at the Ninth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., October 27–29, 1969.

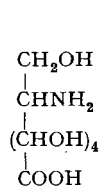
[2]) National Institutes of Health Postdoctoral Fellow, 1967–1970.

[3]) The work on the CMR. spectrum of hygromycin B carried out at the California Institute of Technology was supported by the Public Health Service.

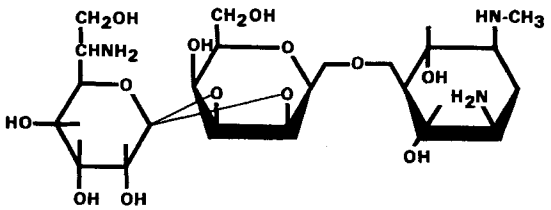
chromatographically homogeneous hygromycin B was obtained. This material was amorphous but gave consistent microanalyses indicating the empirical formula of $C_{20}H_{37}N_3O_{13}$. Treatment of the antibiotic with Ac_2O in pyridine afforded a good yield of crystalline undecaacetate $C_{42}H_{59}N_3O_{24}$. Mass spectrum of this peracetate gave the correct molecular ion at m/e 989. The elemental analysis of undecaacetate indicated the presence of eleven acetylgroups. Three of these are present as N-acetyls as evidenced by the elemental composition of fragments in its high resolution mass spectrum, leaving eight acetyl groups as O-acetyls.

Taking into account the elemental composition of the antibiotic and the earlier findings [2], it became apparent that in addition to eight oxygens present in the molecule of hygromycin B as hydroxyls, two oxygens are present in the B_2 portion (talose and its glycosidic linkage to hyosamine) leaving three oxygen atoms unaccounted for.

A few years ago *Kondo et al.* [3] reported the isolation from a culture broth of *Streptomyces rimofaciens* of another polyhydroxy basic antibiotic, destomycin A, which they found to be isomeric with hygromycin B. Upon hydrolytic cleavage of destomycin A, they obtained D-talose, a levorotatory 2-N-methyl-deoxystreptamine $C_7H_{16}O_3N_2$, a basic levorotatory glucoside $C_{13}H_{26}N_2O_8$ corresponding to the dextrorotatory glucoside of the same composition, named hygromycin B_2, from hygromycin B, and finally a polyhydroxy amino acid $C_7H_{15}NO_7$, designated destomic acid. This last compound was shown to have structure IV. Examination of physical data of all three degradation products as well as chemical correlations led *Kondo et al.* [3] to postulate structure V for destomycin A.



Destomic Acid (IV)



Destomycin A (V)[4]

Hydrolytic cleavage of hygromycin B using 1N hydrochloric acid at 100°C for 10 minutes, followed by adsorption of the reaction mixture on Amberlite IRA resin 400 (OH) and elution with 1% aqueous ammonia, afforded crystalline material. Recrystallization from aqueous methanol yielded destomic acid, $C_7H_{15}O_7N$, m.p. 207–209°; $[\alpha]_D^{25} = +5.2°$ (H_2O, $c = 2$); $[\alpha]_D^{26} = -16.2°$, after 1 hour $-25.9°$ ($c = 2$, 2N HCl) shown to be identical (*X*-ray powder data and NMR. spectra) with an authentic sample of destomic acid[4]) obtained by *Kondo et al.* [3] from destomycin A[4]).

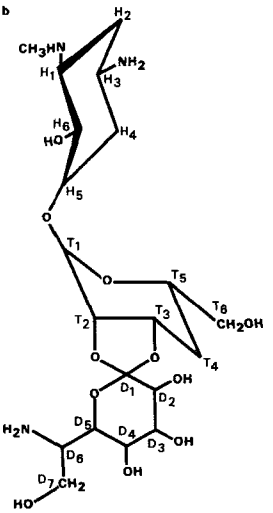
Consideration of these data led to the conclusion that the three oxygens unaccounted for in the molecular formula of hygromycin B and its undecaacetate (*vide supra*) must be present as the ortho ester group involving the attachment of destomic acid moiety to D-talose. The presence of this type of linkage is apparent in the ^{13}C NMR. (CMR.) spectra of hygromycin B and its degradation products.

[4]) We gratefully acknowledge samples of destomycin A and destomic acid from Dr. *Makoto Oda* of *Meiji Seika Kaisha Ltd.*, Kohoku-ku, Yokohama, Japan.

^{13}C Chemical Shift of Hygromycin B, its Degradation Products and Destomycin A[a]

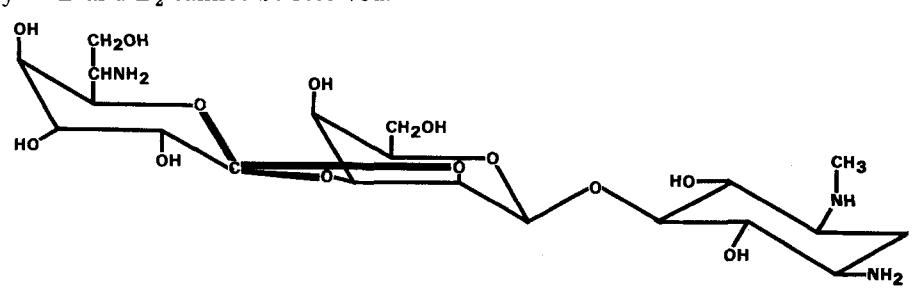
FRAGMENT:	CARBON#	HYOSAMINE	HYGROMYCIN B_2[b]	HYGROMYCIN B[b]	DESTOMYCIN A[b]
	H_1	134.4	134.4	134.4	134.4
	2	160.2	160.5	160.4	160.8
	3	142.0	141.9	141.9	142.0
HYOSAMINE	4	115.0	115.3	115.3	C
	5	116.4	107.1	106.9	106.9
	6	117.3	118.8	118.8	C
	CH_3	160.6	160.5	160.4	160.8
	T_1		91.2	93.5	93.5
	2		121.5*	117.4*	117.2*
	3		123.7*	117.9*	118.2*
TALOSE	4		124.0*	119.2*	118.8*
	5		116.4	120.3*	120.4*
	6		131.2	131.2	131.2
	D_1			73.1	73.2
	2			121.0*	121.0*
	3			123.4*	123.4*
DESTOMIC ACID	4			123.4*	123.4*
	5			128.9*	128.9*
	6			140.6	140.5
	7			130.4	130.5

a) ALL CHEMICAL SHIFTS ARE REFERRED TO EXTERNAL CARBONDISULFIDE AND HAVE UNCERTAINTIES OF ABOUT ±0.1 ppm
b) PEAKS MARKED WITH ASTERISKS CANNOT BE SPECIFICALLY ASSIGNED
c) THE PEAKS HAVE NOT YET BEEN FOUND



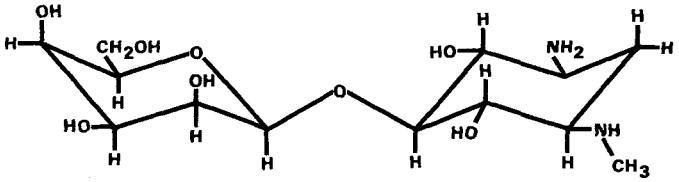
The CMR. spectrum (Table) of hygromycin B consists of 18 well-resolved peaks, one of which (123.4 ppm) is clearly of twice the intensity of the others and therefore represents the overlap of two carbon resonances. Furthermore, the highest field peak is inordinately broad and intense, and for reasons described below is also considered to represent two overlapping resonances. Thus, the CMR. spectrum confirms the presence of twenty carbon atoms in hygromycin B. Using the correlations developed from studies of the inositols [4] and pyranoses [5] it is possible to assign some of these resonances. Thus, the resonance at 93.5 ppm is readily assigned to C-1 of the D-talose fragment, while peaks within the range 130–132 ppm seem likely to represent resonances of hydroxymethyl groups. The lowest field resonance at 73.1 ppm is assigned to the orthoester carbon of the destomic acid moiety and this chemical shift is similar to that of triethyl orthoformate found at 65.6 ppm [6].

The comparison of the CMR. spectra of hygromycin B_2 and hyosamine permits extension of assignments of chemical shifts to resonances of other carbons in the antibiotic (cf. Table). The two high field peaks in the CMR. spectrum of hyosamine are resolved and off-resonance decoupling procedure [7] shows that these can be assigned to the N-methyl and C-2 carbons. These two peaks in the spectra of hygromycin B and B_2 cannot be resolved.

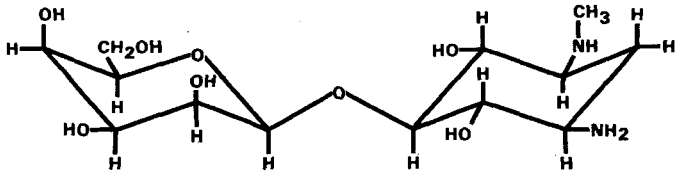


Hygromycin B (VI)

Examination of these data suggested structure VI for hygromycin B[1]). *Wiley et al.* proposed C-5 as the site of attachment of D-talose to hyosamine on the basis of the ease of hydrolysis and similarity of pK_a values of B_2 and hyosamine. Japanese workers suggested β configuration for the basic glycoside VII of destomycin A by the application of *Hudson*'s rule.



Basic Glycoside (from Destomycin A) VII



Hygromycin B_2 (from Hygromycin B) VIII

The corresponding basic glucoside, hygromycin B_2 from hygromycin B, also has the β configuration as evidenced by the NMR. spectrum in $CDCl_3$ of its peracetate. The anomeric proton is found as a broadened doublet at $\delta = 5.25$ ppm. This resonance appears as a sharp doublet, quite typical for β-glycosides [8] in the NMR. spectrum in D_2O of hygromycin B itself at $\delta = 5.72$ ppm, $J = 3$ Hz.

Of particular interest is the nature of amino cyclitols in hygromycin B and destomycin A. Hyosamine is dextrorotatory, $[\alpha]_D^{26} = +40°$ (H_2O). The corresponding amino cyclitol from destomycin A was reported to be levorotatory, $[\alpha]_D^{22} = -17.5°$ ($c = 2$, H_2O). We isolated it from the products of hydrolytic cleavage of destomycin A. Although crystalline, this amino cyclitol still contained some impurities (thin layer chromatography, TLC), $[\alpha]_D^{26} = -26.6°$ (H_2O). The optical rotatory dispersion (ORD.) spectrum of hyosamine displayed the first extremum at 201 nm, $\phi_{201} = +4860°$, whereas the corresponding value for the amino cyclitol from destomycin A was $\phi_{201} = -2370°$. After further purification the amino cyclitol from destomycin A exhibited $[\alpha]_D^{26} = -31.1°$ in H_2O (see Experimental).

Comparison of the CMR. spectra of hygromycin B and destomycin A indicates that the two compounds are closely related. All the specifically assigned resonances of the CMR. spectra of these compounds are within experimental error. However, the two spectra do not match well within the range of chemical shifts at 115–121 ppm; particularly significant in the CMR. spectrum of destomycin A is the absence of two peaks. Unfortunately, the available sample of destomycin A was chromatographically inhomogeneous, and it was impossible to decide whether inconsistencies in the above-mentioned portion of the spectrum arise from impurities in the sample or structural differences between destomycin A and hygromycin B. Possibly the differences could be due to the difference of the amino cyclitol moiety. Unfortunately, we were unable

to get sufficient amounts of the amino cyclitol from destomycin A for its CMR. spectrum.

The different geometry of the amino cyclitol moiety is presumably also responsible for differences in the circular dichroism (CD.) and ORD. of the two antibiotics. The CD. spectrum of hygromycin B has a definite positive maximum at 202 nm; whereas that of destomycin A does not exhibit any maximum up to 190 nm. The ORD. curves of the two antibiotics are dextrorotatory and display a plain positive curve; however, the curve of destomycin A crosses the zero line at about 240 nm and then shows a plain negative curve. It is in this latter respect that its ORD. spectrum differs from that of hygromycin B.

Experimental. – *^{13}C Nuclear Magnetic Resonance Spectra (CMR)* (recorded on a *Varian* DFS-60 using techniques already described [9]), were run in aqueous solution. The chemical shifts are related to 10% internal 1,4-dioxane and expressed in reference to external carbon disulfide by the relation, $\delta(CS_2) = \delta(\text{dioxane}) + 126.1$ ppm.

Proton Magnetic Resonance Spectra (PMR.) were recorded on *Varian* HA-100 MHz instrument. For the spectra to be determined in D_2O, the samples were dissolved in D_2O, and solutions were freeze-dried. The samples were then redissolved in D_2O (10% weight/volume), and the spectra determined. Chemical shifts were measured at 100 MHz relative to Me_4Si as external reference in D_2O solution. The spectra in $CDCl_3$ were measured using Me_4Si as an internal standard.

High Resolution Mass Spectra were recorded using *CEC* high resolution Model 21-110 instrument.

Optical Measurements. ORD. and CD. spectra were recorded on a *Cary* Model 60 recording spectropolarimeter.

Purification of Hygromycin B. A solution of 5 g of crude hygromycin B [1] in 25 ml of water was chromatographed over 800 g of Bio-Rad resin (AG 1 × 2 200–400 mesh) in a column 1100 × 5 cm using water as eluent. Fractions of 200 ml were collected and examined by TLC. on *Merck* cellulose plates using MeOH–$CHCl_3$–NH_4OH (3:1:2 vol.) as solvents and Ninhydrin spray. Fractions No. 35–45 gave 2.8 g of chromatographically pure but amorphous hygromycin B which could not be induced to crystallize. The compound was dried six hours at 120° *in vacuo* for analysis. $[\alpha]_D^{26} = +20.2°$ ($c = 1$, H_2O).

$C_{20}H_{37}N_3O_{13}$	Calc.	C 45.54	H 7.07	N 7.97	O 39.43%
	Found	,, 45.02	,, 7.24	,, 7.69	,, 39,71%

Hygromycin B Undecaacetate. 20 g of crude hygromycin B were stirred in 1 l of a mixture (1:1 vol.) of pyridine and acetic anhydride at room temperature for 72 hours. The solvent was evaporated *in vacuo* and the oily residue taken up in 500 ml of boiling ethyl acetate, filtered and allowed to crystallize. After standing, 25.5 g of crystalline acetate were obtained, m.p. 285° (dec.). For analysis the compound was dried on the block for 1 minute at 150°.

$C_{42}H_{59}N_3O_{24}$	Calc.	C 50.96	H 6.01	N 4.24	acetyl (11) 47.7%	M^+ 989.3519
	Found	,, 50.75	,, 6.27	,, 4.32	,, 47.7%	,, 989.3488[5])

Preparation of Hyosamine from Hygromycin B. A solution of 5 g of hygromycin B in a mixture containing 50 ml of H_2O and 50 ml of 12 N HCl was refluxed for 16 hours. After cooling and filtration the mixture was extracted six times with Et_2O and the aqueous phase concentrated to dryness. After redissolving in 150 ml of H_2O, 1.5 g of decolorizing carbon was added and the suspension filtered. A solution of 11.5 g of picric acid in 600 ml of H_2O was prepared with heating and filtered after cooling to room temperature. The two solutions were combined and allowed to stand overnight at 0°. After filtration, the solids (ca. 4.0 g) were dissolved in boiling water, allowed to crystallize, and the picrate filtered off. The resulting mass (ca. 3.09 g) was suspended in 40 ml of H_2O and in 25 ml of AG 1 × 4 (OH) washed resin, and stirred for one hour. The solids were separated by filtration and washed with water. The filtrate was evaporated to dryness and the residue

[5]) High Resolution Mass Spectrum.

recrystallized from 15 ml of hot absolute ethanol. After standing overnight in refrigerator, 0.85 g of colorless hysoamine, m.p. 183–186°, were obtained. $[\alpha]_D^{26} = +39.8°$ ($c = 2$, H_2O).

$C_7H_{16}N_2O_3$ Calc. M^+ 176.1189 Found M^+ 176.1205[5])

Preparation of N-Methyl-deoxystreptamine from Destomycin A. This amino cyclitol was prepared analogously to hyosamine; however, starting from 5 g of crude destomycin A, only 210 mg of crystalline, chromatographically impure material were obtained. This was first chromatographed on Bio-Rad resin (150 g, Dowex, 200–400 mesh, OH cycle) using water as eluent yielding 180 mg of still impure amino cyclitol $[\alpha]_D^{26} = -26.6°$ (H_2O) (TLC., *Merck* Cellulose Plates, MeOH–$CHCl_3$–NH_4OH 3:1:2 vol.). This material was rechromatographed on 25 g of cellulose (*Schleicher & Schuell*, grade 286) using MeOH–$CHCl_3$–NH_4OH 3:1:2 vol. solvent mixture. Fractions of 15 ml were collected and examined by TLC. Fractions No. 1–7 were discarded. Fraction No. 8 gave 119 mg of crystalline, chromatographically homogeneous amino cyclitol melting at 120–126°, then resolidifying and melting at 160–162°. $[\alpha]_D^{26} = -31{,}1°$ ($c = 1$, H_2O).

$C_7H_{16}N_2O_3$ Calc. M^+ 176.1189 Found 176.1160[5])

Preparation of Destomic Acid from Hygromycin B. This acid was prepared analogously to procedure described for its isolation from destomycin A [3]. The material was identical (IR., NMR., *X*-ray powder data and TLC.) with an authentic sample from the Japanese investigators[4]). M.p. 207–209°. $[\alpha]_D^{26} = +5.3°$ ($c = 2$, H_2O).

We thank the following members of Molecular Structure Research Division of the *Lilly Research Laboratories* for recording physical and analytical data: Messrs. *T. K. Elzey* – PMR. spectra; *J. P. Hettle* – mass spectra; *G. M. Maciak* and his associates – microanalyses; *F. V. Beasley* – ORD. and CD. spectra.

BIBLIOGRAPHY

[1] *R. L. Mann & W. W. Bromer*, J. Amer. chem. Soc. *80*, 2714 (1958).
[2] *P. F. Wiley, M. V. Sigal, Jr. & O. Weaver*, J. org. Chemistry *27*, 2793 (1962).
[3] *S. Kondo et al.*, J. of Antibiotics *18*, 38, 192 (1965); *19*, 137, 139 (1966).
[4] *D. E. Dorman, S. J. Angyal & J. D. Roberts*, Proc. Nat. Acad. Sci. *63*, 612 (1969); J. Amer. chem. Soc. *92*, 1351 (1970).
[5] *D. E. Dorman & J. D. Roberts*, J. Amer. chem. Soc. *92*, 1355 (1970).
[6] *J. W. Emsley, J. Feeney & L. H. Sutcliffe*, High Resolution Nuclear Magnetic Resonance Spectroscopy, Vol. 2, p. 995, Pergamon, London 1966.
[7] *J. D. Roberts, F. J. Weigert, J. I. Kroschwitz & H. J. Reich*, J. Amer. chem. Soc. *92*, 1338 (1970).
[8] *R. V. Lemieux, R. K. Kulling, H. J. Bernstein & G. W. Schneider*, J. Amer. chem. Soc. *80*, 6098 (1958).
[9] *F. J. Weigert & J. D. Roberts*, J. Amer. chem. Soc. *89*, 2967 (1967); *F. J. Weigert, M. Jautelat & J. D. Roberts*, Proc. Natl. Acad. Sci. *60*, 1152 (1968).

---

276. Equilibres d'ionisation et photoréduction du vanadium(V) dans l'acide sulfurique concentré

par **M. Marcantonatos, R. Piguet** et **D. Monnier**

Institut de Chimie minérale et de Chimie analytique de l'Université de Genève

(10 VI 70)

Summary. By spectrophotometry of dilute solutions of V_2O_5 in concentrated sulfuric acid V^V–H_2SO_4 complexes are shown to convert slowly to V^{IV} monomers. Spectral, kinetic and equilibrium studies on these solutions suggest that this conversion involves a photoreduction of V^V to V^{IV} dimeric species.