δ 8.2–6.8 (m, 8 H, arom), 3.80 (s, 3 H, OCH_3), 3.77 (s, 2 H, CH_2). *Anal.* ($C_{17}H_{13}NO$) C, H.

2-Hydroxy-11*H*-indeno[1,2-*b*]quinoline (12).—To a stirring hot sol of 1.20 g of **11** in 35 ml of glacial HOAc was slowly added 25 ml of 48% HBr. The resulting yellow soln was refluxed and stirred for 45 hr and cooled, and the product was collected by vacuum filtration. The microcryst needles thus obtd were washed several times with H_2O and dried giving 1.48 g (97%) of **12**·HBr which sublimes but does not melt below 320°. Recrystn from *i*-PrOH–MeOH afforded the analytic sample. *Anal.* ($C_{16}H_{11}NO$·HBr) C, H.

The free base was obtained by dissolving the hydrobromide in 1 *N* NaOH and pouring the basic soln into coned NH_4Cl. Filtration and recrystn from EtOH–H_2O gave cryst **12**: mp 299–301° dec; ir (KBr) 3525 (phenolic OH) cm^{-1}; mass spectrum *m/e* 233 (base, M^+). *Anal.* ($C_{16}H_{11}NO$) C, H.

2-Hydroxy-10-chloro-11*H*-indeno[1,2-*b*]quinoline (13) was prepd from **9** in 92% yield as described for **12**. Recrystn of **13**·HBr from DMF gave pure material: mp >320° subl. *Anal.* ($C_{16}H_{10}ClNO$·HBr) C, H.

Acknowledgment.—The author wishes to express his appreciation to Dr. H. B. Wood, Drug Development Branch, National Cancer Institute, for his interest in this work and for making the screening data available.

Gentamicin Antibiotics. 4.[1] Some Condensation Products of Gentamicin C_2 with Aromatic and Aliphatic Aldehydes[2]

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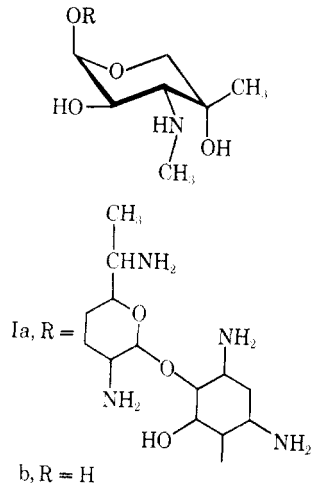
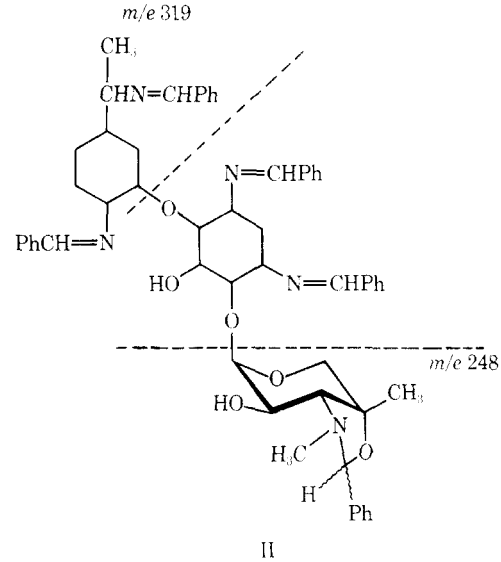
Received September 27, 1970

The gentamicins are a family of broad-spectrum antibiotics belonging to the aminoglycoside group. The isolation,[3] biological properties,[4a–d] and chromatographic separation[5a,b] of the gentamicins have been published and a recent communication[6] from our laboratory has described the gross structures of the components of the gentamicin C complex. One member of the complex, gentamicin C_2 was shown to possess structure Ia. In common with the other gentamicins, Ia is not absorbed to any great extent when given orally in man and, given parenterally, it is rapidly excreted in the urine requiring relatively frequent dosing in order to maintain effective blood levels. It seemed reasonable that a lipophilic derivative of the antibiotic, from which the parent compound could be regenerated *in vivo*, might provide oral absorption or longer duration of action when given parenterally. Such a derivative could be formed by condensation of the primary amino groups with aldehydes, a procedure described previously for the related antibiotic kanamycin.[7]



Gentamicin C_2 reacted readily with BzH in EtOH on gentle heating to give a colorless, crystalline benzylidene derivative. Microanal. of this material indicated that 5 aldehyde groups—not 4 as expected—had been incorporated into the molecule. Low-resolution mass spectrometry of this compound surprisingly gave a strong molecular ion at *m/e* 903 consistent with condensation of 5 aldehyde residues with elimination of 5 moles of H_2O. The recent determination of the L-arabino absolute stereochemistry for garosamine[8a,b] (Ib) enables this result to be interpreted in terms of the formation of the novel oxazolidine (II). This was substantiated by the appearance in the mass spectrum of intense peaks at *m/e* 319 (cleaved purpurosamine fragment) and *m/e* 248 (cleaved garosamine fragment). Formation of the oxazolidine was confirmed by examination of the ^1H nmr spectrum (60 MHz, $CDCl_3$) which contained a 1-proton singlet at δ 5.0 corresponding to the benzylic proton of the oxazolidine system and indicating further that oxazolidine formation proceeds stereospecifically.



II

(1) Part 3: D. J. Cooper, M. D. Yudis, H. M. Mangliano, P. J. L. Daniels, R. D. Guthrie, and S. T. Bukhari, submitted for publication.

(2) Presented in part before the Division of Medicinal Chemistry, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, MEDI 052.

(3) M. J. Weinstein, G. M. Luedemann, E. M. Oden, and G. H. Wagman, *Antimicrob. Ag. Chemother.*, 1 (1963).

(4) (a) M. J. Weinstein, G. H. Wagman, E. M. Oden, and J. A. Marquez, *J. Bacteriol.*, **94**, 789 (1967); (b) J. Black, B. Calesnick, D. Williams, and M. J. Weinstein, *Antimicrob. Ag. Chemother.*, 138 (1963); (c) M. J. Weinstein, G. M. Luedemann, E. M. Oden, and G. H. Wagman, *ibid.*, 816 (1965); (d) J. A. Waitz and M. J. Weinstein, *J. Infect. Dis.*, **119**, 355 (1969).

(5) (a) G. H. Wagman, J. A. Marquez, and M. J. Weinstein, *J. Chromatogr.*, **34**, 210 (1968); (b) H. Maehr and C. P. Schaffner, *ibid.*, **30**, 572 (1967).

(6) D. J. Cooper, H. M. Marigliano, M. D. Yudis, and T. Traubel, *J. Infect. Dis.*, **119**, 342 (1969).

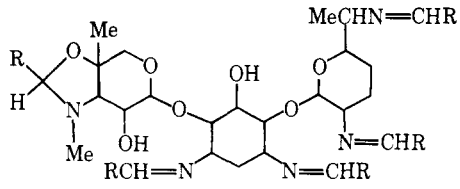
(7) A. Fujii, K. Maeda, and H. Umezawa, *J. Antibiot.*, **21**, 340 (1968).

(8) (a) D. J. Cooper, M. D. Yudis, R. D. Guthrie, and A. M. Prior, *J. Chem. Soc. C*, 960 (1971); (b) W. Meyer zu Reckendorf and E. Bischof, *Tetrahedron Lett.*, 2375 (1970).

Gentamicin C_2 was treated with 7 other aldehydes to give, in each case, a derivative corresponding to the benzylidine compound. These compounds are shown in Table I and all possess *in vitro* antibacterial activity

TABLE I

ALDEHYDE CONDENSATION PRODUCTS FROM GENTAMICIN C_2

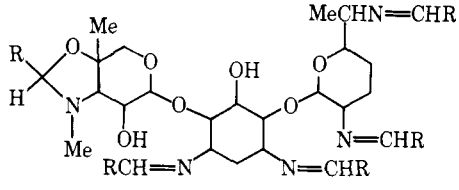


R	Mp, °C	$[\alpha]^{26}D$ (c, 0.3% $CHCl_3$), deg
C_6H_5	206–212	−16.8
2,6-$Cl_2C_6H_3$	108–123	+15.4
5-NO_2-2-furfuryl	>325	−21.7
C_6F_5	211–217	+34.4
p-$NO_2C_6H_4$	163–168	−42.0
p-ClC_6H_4	173–177	−37.0
n-$C_{11}H_{23}$	63–65	+80.3
α-Naphthyl	187–191	+41.3

comparable to the parent gentamicin, presumably because they can undergo facile hydrolysis to the parent antibiotic. Minimum inhibitory concns were determined in yeast beef broth at pH 6.8 and are shown for representative compounds in Table II. It is evident

TABLE II

In Vitro ANTIBACTERIAL ACTIVITY IN YEAST BEEF BROTH AT pH 6.8



	MIC, μg/ml			
R	*Entero-coccus* sp. DA800	*E. coli* ATCC 10536	*P. aeru-ginosa* ATCC 8709	*S. aureus* ATCC 6538P
Gentamicin C_2	3.0	0.3	0.3	0.03
C_6H_5	0.75	0.3	0.3	0.3
5-NO_2-2-furfuryl	3.0	0.3	0.75	0.03
C_6H_5	0.75	0.03	0.3	0.03
p-ClC_6H_4	0.3	0.3	0.75	0.3
α-Naphthyl	3.0	0.3	0.3	0.03

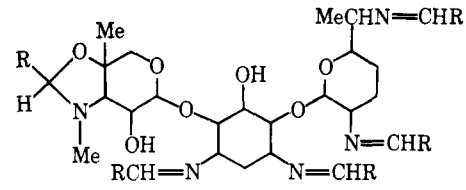
that the spectrum of activity and potency is similar to that of the parent antibiotic.

Serum levels were determined in mice treated sc with a suspension of each compound in 0.5% aq CM-cellulose sonicated to reduce particle size. The results are shown in Table III; in general all of the compounds produced lower serum levels than the parent antibiotic, however there is some evidence of prolonged duration of action in the cases of the nitrofuryl and dodecyl derivatives. In these cases peak serum levels occurred at a later time than in the case of gentamicin C_2 and measurable serum levels were still present after 6 hr.

Oral absorption studies were also conducted using cannulated dogs. The compounds were administered

TABLE III

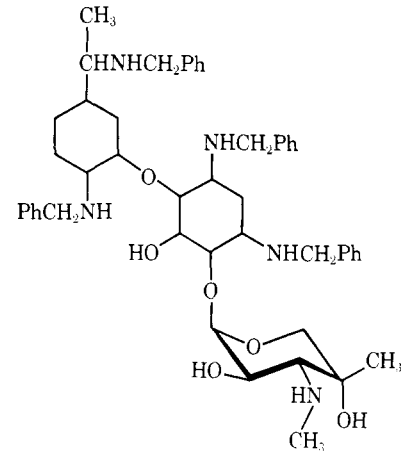
SERUM LEVELS (ASSAYED AS GENTAMICIN C_2) IN MICE TREATED WITH 25 mg/kg SC AS A SONICATED SUSPENSION IN 0.5% AQ CM-CELLULOSE



	Serum levels, μg/ml — Time after dosing, hr					
R	0.25	0.5	1	2	4	6
Gentamicin C_2 sulfate	26.0	15.0	5.0	0.5	<0.04	0
C_6H_5	0.9	0.7	0.4	0	0	0
5-NO_2-2-furfuryl	1.6	1.8	1.2	0.7	0.4	0.6
C_6F_5	1.1	0.9	1.4	0.3	<0.04	0
p-ClC_6H_4	1.4	1.3	0.5	0.3	<0.04	0
α-Naphthyl	0.7	0.4	0.4	<0.04	0	0
n-$C_{11}H_{23}$	0.9	0.8	0.8	0.6	0.6	0.5

intraduodenally as suspensions in aq 0.5% CM-cellulose to avoid acid hydrolysis in the stomach. There were no detectable serum levels produced. In oral protection and absorption studies using mice, the aldehyde condensation products were, without exception, less effective than gentamicin C_2 sulfate.

Reduction of the benzaldehyde condensate with $NaBH_4$ in EtOH or PrOH and subsequent acid work-up afforded the tetra-*N*-benzyl derivative III which proved



III

to be biologically inactive. Similar treatment of the remaining oxazolidine–Schiff bases with $NaBH_4$ resulted in every case in loss of biological activity, although no attempt was made to isolate the resulting *N*-alkyl compounds.

Experimental Section

Nmr spectra were detd on a Varian A60A spectrometer (60 MHz), and mass spectra on a Perkin Elmer-Hitachi RMU-6 spectrometer using the direct inlet system. Melting points were detd on a Kofler apparatus and are uncor. Tlc was performed on 20 × 10 cm silica gel plates and the compounds were visualized with I_2 vapor. Column chromatography utilized chromatographic grade silica gel (J. T. Baker and Co.) and was performed on high-resolution columns obtained from Glenco (Houston).

Aldehyde Condensation Products of Gentamicin C_2.—Gentamicin C_2 (0.001 *M*) was dissolved in EtOH (25 ml), aldehyde (0.005 mole) was added, and the soln was refluxed for 1 hr. Purification was then effected by one of 2 methods. (a) When the

product formed a ppt it was filtered off and recrystd from either C_6H_6 or $CHCl_3$–hexane. (b) When the product remained in soln the EtOH was removed *in vacuo* and the resulting foam was dissolved in $CHCl_3$ and added dropwise, with stirring to 10 vol of hexane. The ppt was filtered off and further purified by a second pptn. Yields varied between 50 and 80%.

Tetrabenzylgentamicin C_2 (III).—$NaBH_4$ (0.43 g) was added to a refluxing soln of pentabenzylidine gentamicin C_2 (0.66 g) in abs EtOH (25 ml). Refluxing was contd for 3.5 hr. The soln was cooled and acidified to pH 1.5 with 1 *N* H_2SO_4–MeOH. After 1 hr at room temp the soln was poured into dil NH_4OH (2 *N*, 200 ml) and extd with $CHCl_3$ (2 × 100 ml). The ext was washed with 5% $NaHSO_3$ and H_2O, dried ($MgSO_4$), filtered, and evapd to yield a colorless foam. Tlc (silica gel, $CHCl_3$–MeOH, 9:1) showed 1 major component, R_f ~0.7, and several minor impurities. Column chromatography on silica gel (45 g) in the same system afforded the pure tetra-*N*-benzylgentamicin C_2 as a colorless foam (0.35 g): mp 63–65°, $[\alpha]^{25}D$ + 80.3° (0.3, $CHCl_3$). *Anal.* ($C_{48}H_{65}N_5O_7$) calcd, C, 69.9; H, 7.97; N, 8.50%. Found, C, 70.6; H, 7.63; N, 7.48. It did not prove possible to obtain a better microanal. and this we attribute to occluded solvent, a problem we have encountered with several other free base aminoglycosides.[9]

(9) Part 2: D. J. Cooper, M. D. Yudis, H. M. Marigliano, and T. Traubel, *J. Chem. Soc.*, submitted for publication.

Catalytic Hydrogenation of Viomycin and Capreomycin[1]

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Received April 23, 1971

Viomycin, isolated in 1950 from cultures of *Streptomyces puniceus* and *Streptomyces floridae*,[2] and capreomycin, isolated in 1960 from cultures of *Streptomyces capreolus*,[3] are well-known antibiotics used against streptomycin-resistant strains of *Mycobacterium tuberculosis*. Both cause serious side effects, including kidney damage, vestibular dysfunction, electrolyte imbalance, and hypersensitivity. New derivatives of viomycin and capreomycin that demonstrate biological activity are of interest since any derivative that has fewer or less serious side effects would be important in the treatment of disease.

The perhydrogenated derivatives of both viomycin and capreomycin were suggested by a brief but undetailed report of the catalytic reduction of viomycin.[4] Both viomycin and capreomycin were catalytically hydrogenated, and the purity and properties of the resulting products were studied. Tlc in several solvent systems revealed that the hydrogenated materials were not identical with the starting materials. Both perhydrogenated materials showed only one ninhydrin-positive spot in every solvent system tried. Urea was found in the crude perhydrocapreomycin mixture, but not in the perhydroviomycin preparation.

Although viomycin shows strong absorption in the uv region (λ_{max} 268 (ϵ 23,000) in 0.1 *N* HCl and λ_{max} 282.5 nm (ϵ 14,600) in 0.1 *N* NaOH), perhydroviomycin showed only end absorption in both solvents. Perhydrocapreomycin showed absorption at λ_{max} 256 ($E^{1\%}_{1cm}$ 78) and λ_{max} 263 nm ($E^{1\%}_{1cm}$ 74) in H_2O, in contrast to the absorption of capreomycin complex itself, λ_{max} 266 ($E^{1\%}_{1cm}$ 260) in H_2O.

The nmr spectra of both perhydroviomycin and perhydrocapreomycin were similar to the spectra of the parent compounds except that in each case, the absorption at τ 1.9 in both viomycin and capreomycin had disappeared.

The biological activities of perhydroviomycin and perhydrocapreomycin were measured by the zone inhibition method using *Bacillus subtilis*. Perhydroviomycin was found to be approximately 30% as active as viomycin, and perhydrocapreomycin 43% as active as capreomycin.

In order to demonstrate homogeneity, the perhydroviomycin was chromatographed using a 550 cm × 1.9 cm Sephadex G-15 column. A total recovery of 92% was achieved from the column. A wt curve of individual fractions was plotted, which revealed that 93% of the material recovered was homogeneous, giving a Gaussian peak in the curve. Bioassay of each fraction in the peak revealed an average activity of 30% of the activity of commercial viomycin.

Experimental Section

Perhydroviomycin.—A 6.8-g sample of dried commercial viomycin sulfate was dissolved in 50 ml of 50% aq AcOH. A 10% Pt–C catalyst (7.5 g) was slurried with 250 ml of 50% aq AcOH and equilibrated with H_2. The viomycin sample was introduced with careful exclusion of air, and the soln was stirred under H_2 at room temp and atm pressure for 410 hr. A total of 520 ml of H_2 (STP) was absorbed. The catalyst was filtered off through a bed of Celite, and the filtrate was lyophilized to give 7.3 g of a fluffy, white, amorphous powder. This material was stirred with 90 ml of IR-45(OH^-) ion-exchange resin until the pH was 5.5, the resin was removed by filtration, and the filtrate was applied to a column contg 60 ml of IR-45(SO_4^{2-}) resin and eluted with 250 ml of H_2O. The eluate was lyophilized to give 6.2 g of perhydroviomycin as the stoichiometric sulfate salt. The uv spectrum of this material showed no absorption except end absorption. Tlc in H_2O, 1-BAW,[5] and 1-BAWAA[5] revealed only one ninhydrin-positive spot, which was different from viomycin in R_f value. Bioassay by the zone inhibition method using *B. subtilis* revealed the perhydroviomycin to be 30% as active as viomycin itself.

A 2.4-g sample of the perhydroviomycin was chromatographed on a 550 cm × 1.9 cm Sephadex G-15 column that had been equilibrated with 0.01 *N* HCO_2H. A total of 2.2 g of this material (92.4%) was recovered. A wt curve of the individual fractions was plotted, which showed that 93% of the material recovered was homogeneous and gave a Gaussian peak in the curve. Bioassay of each fraction in the peak revealed an average activity of 30% of the activity of commercial viomycin.

Perhydrocapreomycin.—A 4.5-g sample of dried commercial capreomycin was treated in the same manner as the viomycin except that the soln was stirred under H_2 for 500 hr. A total of 368 ml of H_2 (STP) was absorbed. The hydrogenated material was worked up in the same manner as perhydroviomycin. The uv spectrum of the perhydrocapreomycin showed absorptions at λ_{max} 256 ($E_{1cm}^{1\%}$ 78) and λ_{max} 263 nm ($E_{1cm}^{1\%}$ 74) in H_2O.

(5) 1-BAW is 1-BuOH–AcOH–H_2O (4:5:1); 1-BAWAA is 1-BuOH–AcOH–H_2O–Me_2CO–3 *N* NH_4OH (9:2:4:3:2).

(1) This investigation was supported in part by a National Institute of Health Grant.

(2) (a) A. J. Finlay, G. L. Hobby, F. Hochstein, T. N. Lees, T. F. Lenert, J. A. Means, S. Y. P'An, P. P. Regna, J. B. Routien, B. A. Sobin, K. B. Tate, and J. H. Kane, *Amer. Rev. Tuberc.*, **63**, 1 (1951); (b) Q. R. Bartz, J. Ehrlich, J. D. Mold, M. A. Penner, and R. M. Smith, *ibid.*, **63**, 4 (1951); (c) W. S. Marsh, R. L. Mayer, R. P. Mull, C. R. Scholz, and R. W. Townley, U. S. Patent 2,633,445 (1953) *Chem. Abstr.*, **47**, 8974c (1953); (d) T. G. Freaney, U. S. Patent 2,828,245 (1958); *Chem. Abstr.*, **52**, 12333e (1958).

(3) (a) E. B. Herr, Jr., M. E. Haney, G. E. Pittenger, and C. E. Higgins, *Proc. Indiana Acad. Sci.*, **96**, 134 (1960); (b) E. B. Herr, R. L. Hamill, and J. M. McGuire, U. S. Patent 3,143,468 (1964); *Chem. Abstr.*, **61**, 11297a (1965). (c) British Patent 920,563 (1963); *Chem. Abstr.*, **60**, 1078e (1964).

(4) (a) B. W. Bycroft, D. Cameron, L. R. Croft, A. W. Johnson, T. Webb, and P. Coggen, *Tetrahedron Lett.*, 2925 (1968); (b) B. W. Bycroft, D. Cameron, L. R. Croft, A. Hassanali-walji, A. W. Johnson, and T. Webb, *ibid.*, 5901 (1968).