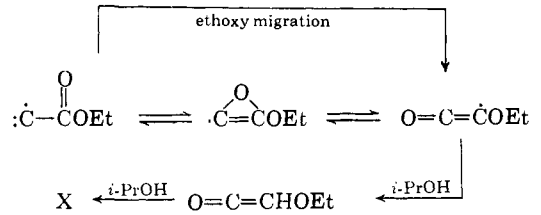
oxygen since under anerobic conditions their yield fell drastically. The two principal products VI and VII can then be rationalized as being formed by a C–H insertion and OH insertion or polar addition of carbethoxymethyne, followed by H abstraction. Product XI may form from dehydration of VI. Of particular interest is product X with a net shift of either the ethoxy group or of the carbonyl oxygen. The process may be envisaged as going through a ketene type intermediate.[7]



Again, as with cyclohexene, the product distribution was significantly different from that obtained from the reaction of carbethoxymethylene, indicating that the latter is not a principal transient in the present reaction. (Photolysis of ethyl diazoacetate in 2-propanol gave rise to VI, VII, X, and XII in a ratio of 9:25:29:12).[7]

The insertion reactions of carbethoxymethyne appear to be highly selective, which may partly be attributed to the intervention of polar structures in the transition state analogous to the one postulated by Doering and Knox for carbethoxymethylene.[8]

Further extensive studies on a series of monovalent carbon intermediates, including species like NC–Ċ:, RCOĊ:, PhCOĊ:, CF_3Ċ:, and :ĊH itself, are currently underway in our laboratory.

The results of a preliminary esr study of $(CF_3CN_2)_2Hg$ and the photolysis of $(PhCOCN_2)_2Hg$ are in good agreement with the proposed intervention of a carbyne intermediate in the photodecomposition of the diazomercurial structure.

Product identifications in the present study were accomplished by high-resolution mass spectrometry, infrared spectra, and nmr spectroscopy in conjunction with unambiguous syntheses and chemical transformations to known derivatives.

Acknowledgment. The authors are grateful to the National Research Council of Canada for financial support.

(7) Carbethoxymethylene has been shown to undergo an analogous rearrangement to yield carbethoxy ketene: T. DoMinh, O. P. Strausz, and H. E. Gunning, submitted for publication.

(8) W. von E. Doering and L. H. Knox, *J. Am. Chem. Soc.*, 83, 1989 (1961).

Thap DoMinh, H. E. Gunning, O. P. Strausz
Department of Chemistry, University of Alberta
Edmonton, Alberta, Canada
Received July 5, 1967

The Chemistry of the Gentamicins. I. Characterization and Gross Structure of Gentamicin A

Sir:

Gentamicin A, a constituent of the gentamicin complex,[1] is a broad-spectrum antibacterial antibiotic.[2] Its separation from the commercial gentamicin C complex as a coproduced antibiotic[1] and its chromatographic differentiation from other gentamicins and related antibiotics[3] have been described.

Crude gentamicin A can be purified as the N-acetylated derivative by liquid–liquid partition chromatography followed by regeneration to the free base with alkaline hydrolysis.[4] Fractional crystallization from aqueous methanol afforded pure gentamicin A free base as colorless clusters of prismatic needles, $[\alpha]^{23}D$ +136° (*c* 1.0, H_2O).

Thin layer chromatography[5] reveals only one component. Crystalline gentamicin A free base has a constant weight at room temperature and 2 mm. *Anal.* Calcd for $C_{18}H_{36}N_4O_{10}\cdot 1.5H_2O$: C, 43.63; H, 7.93; N, 11.31; O, 37.13. Found: C, 43.60; H, 7.86; N, 11.56; O, 37.16. The base hydrate sinters at 151–154° with loss of crystal water and decomposes slowly above 200°. The quantitative removal of the crystal water is accomplished with great difficulty. *Anal.* Calcd for $C_{18}H_{36}N_4O_{10}$: C, 46.15; H, 7.75; N, 11.96; O, 34.15; mol wt, 468.5. Found (after drying to constant weight at 120° *in vacuo*): C, 45.70; H, 7.60; N, 11.80; O, 34.90; mol wt, 410 (Signer). Conversion to the N-acetyl derivative produced a homogeneous substance.[6] *Anal.* Calcd for $C_{18}H_{32}N_4O_{10}(COCH_3)_4$: acetyl, 27.04. Found: acetyl, 26.44 (hydrazinolysis). Gentamicin A free base is soluble in water and partly soluble in methanol but practically insoluble in ethanol and less polar solvents. Gentamicin A exhibits positive ninhydrin, Elson–Morgan, and Lemieux–Bauer reactions, but negative Sakaguchi and Fehling reactions. In contrast to kanamycin, neomycin, and paromomycin, gentamicin A does not yield a furfural-type of chromophore when treated with 40% sulfuric acid.

Hydrolysis of gentamicin A in 6 *N* hydrochloric acid for 6 hr at 100° results in complete destruction of biological activity. Paper chromatography[7] of the hydrolysate and ninhydrin development revealed the presence of four major components. The slowest component (R_f 0.11) was obtained in crystalline form after charcoal decolorization, concentration to a syrup, and addition of methanol and ethanol. After recrystallization it was found to be identical with 2-deoxystreptamine dihydrochloride isolated from neomycin,[8] kanamycin,[9] and paromomycin.[10] Identity was confirmed by paper chromatography, infrared spectroscopy, and optical rotation measurement. A second crystalline hydrolysis product (R_f 0.26) obtained from the mother liquor was identified as 2-amino-2-deoxy-D-glucose hydrochloride by paper chromatography, infrared spectroscopy,

(1) M. J. Weinstein, G. M. Luedemann, E. M. Oden, and G. H. Wagman, "Antimicrobial Agents and Chemotherapy," American Society for Microbiology, Ann Arbor, Mich., 1963, p 1.

(2) M. J. Weinstein, G. M. Luedemann, E. M. Oden, and G. H. Wagman, "Antimicrobial Agents and Chemotherapy," American Society for Microbiology, Ann Arbor, Mich., 1965, p 816.

(3) H. Maehr and C. P. Schaffner, *J. Chromatog.*, **30**, 572 (1967).

(4) G. H. Wagman and M. J. Weinstein, *J. Med. Chem.*, 7, 800 (1964).

(5) Silica gel G; chloroform–methanol–28% ammonium hydroxide–water, 1:4:2:1, v/v; R_f 0.60.

(6) Descending paper chromatography, Whatman No. 1; 1-butanol–pyridine–water, 3:2:2, v/v; R_f 0.30.

(7) Descending paper chromatography, Whatman No. 1; 1-butanol–pyridine–water–acetic acid, 6:4:3:1, v/v.

(8) D. A. Kuehl, Jr., M. N. Bishop, and K. Folkers, *J. Am. Chem. Soc.*, **73**, 881 (1951).

(9) M. J. Cron, D. L. Johnson, F. M. Palermiti, Y. Perron, H. D. Taylor, D. F. Whitehead, and I. R. Hooper, *ibid.*, **80**, 752 (1958).

(10) T. H. Haskell, J. C. French, and Q. R. Bartz, *ibid.*, 81, 3480 (1959).

oxidative degradation to ribose,[11] and optical rotation measurement. The third hydrolysate component (R_f 0.30) was not reducing with aniline hydrogen phthalate, whereas the fourth constituent (R_f 0.39) was reducing. The latter two substances could not be identified as constituents common to known 2-deoxystreptamine-containing antibiotics.

The isolation of 2-deoxystreptamine and 2-amino-2-deoxy-D-glucose suggests by biogenetic reasoning the presence of α-2-amino-2-deoxy-D-glucopyranosyl-(1→4)-1,3-diamino-1,2,3-trideoxy-*scyllo*-inositol,[12,13] also known as pseudoneamine[14] or paromamine.[10,15] Therefore, anhydrous gentamicin A was refluxed in methanolic hydrogen chloride for 36 hr. The crystalline precipitate was collected and recrystallized repeatedly from aqueous ethanol, yielding white needles, $[\alpha]^{23}D$ +82° (*c* 0.5, H_2O), R_f 0.10.[7] *Anal.* Calcd for $C_{12}H_{25}N_3O_7 \cdot 3HCl \cdot H_2O$: Cl, 23.60. Found: Cl, 23.39. Conversion to the free base was followed by crystallization from aqueous ethanol, $[\alpha]^{23}D$ +110° (*c* 0.9, H_2O). *Anal.* Calcd for $C_{12}H_{25}N_3O_7$: C, 44.57; H, 7.79; N, 13.00; O, 34.64. Found: C, 44.56; H, 7.96; N, 12.78; O, 34.96. Hydrolysis in 6 *N* hydrochloric acid at 100° was complete after 6 hr and afforded only 2-deoxystreptamine and 2-amino-2-deoxy-D-glucose, the rate of disappearance of starting material being equal to that of authentic paromamine trihydrochloride. Final proof of identity with paromamine was provided by comparison of infrared spectra and by paper chromatography[7] of the trihydrochlorides.

From the mother liquor of the methanolysate, the crystalline anomeric mixture of methyl gentosaminide free bases was isolated after conversion over Amberlite IRA 400 in the hydroxide form and crystallization from a mixture of methanol, ethanol, and acetone; R_f 0.54.[7] *Anal.* Calcd for $C_6H_{12}NO_3(OCH_3)$: C, 47.45; H, 8.53; N, 7.90; O, 36.12; OCH_3, 17.51. Found: C, 47.63; H, 8.48; N, 8.08; O, 36.05; OCH_3, 16.81. The nmr spectrum was obtained in deuterium oxide solution with TMS as external standard, confirming the presence of O-methyl by a singlet at τ 6.54 and suggesting a N-methyl group by an equally intense signal at τ 7.55.

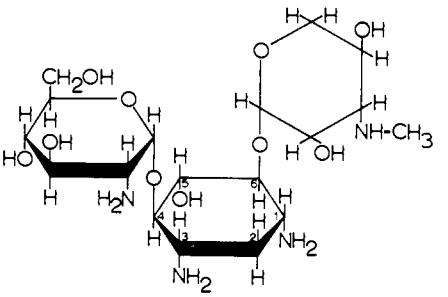
Methyl gentosaminide gives positive ninhydrin and Pan–Dutcher reactions; 1 mole reduced 2 moles of periodate accompanied by the liberation of 1 mole of methylamine. Methylamine was identified by tlc[16] and by its dinitrophenyl derivative, which did not depress the mixture melting point at 178° involving authentic N-methyl-2,4-dinitroaniline.[17]

The anomeric mixture of methyl gentosaminide bases was converted to crystalline N-acetyl derivatives. *Anal.* Calcd for $C_9H_{17}NO_5$: C, 49.31; H, 7.82; N, 6.39; O, 36.49. Found: C, 49.24; H, 7.79; N, 6.45; O, 36.61. This substance gave a negative Pan–Dutcher reaction, indicating the tertiary nature of the nitrogen atom. Its resistance toward periodate completes the preliminary characterization of methyl gentosaminide as methyl 3-methylamino-3-deoxypentopyranoside.

Methanolysis of gentamicin A yielded paromamine and the anomeric methyl gentosaminides as the only major products. Refluxing methyl gentosaminide in 1 *N* sulfuric acid for 24 hr produced mostly reducing gentosamine, R_f 0.39,[7] and smaller amounts of a nonreducing compound, R_f 0.30,[7] both constituents of gentamicin A hydrolysates. Gentosamine refluxed in acid solution produces the nonreducing substance; in methanolic hydrogen chloride methyl gentosaminide is reproduced.

The stereochemistry of gentosamine and the nature of the nonreducing compound, probably anhydrogentosamine, are under investigation.

One mole of gentamicin A reduced 4 moles of periodate. The periodate oxidation product was treated with 48% hydrobromic acid for 5 hr at 100° to furnish 2-deoxystreptamine in 90% yield. Similar results were obtained with kanamycin A, whereas 2-deoxystreptamine was completely destroyed in a neomycin B control. Therefore, the gentosamine moiety in gentamicin A exists in the pyranosyl form and is linked to C_6 of 2-deoxystreptamine.



These experimental findings permit the assignment of the above structure to gentamicin A.

Acknowledgment. We wish to acknowledge in part the support of the National Institute of Allergy and Infectious Diseases under Public Health Service Grant AI-06182. We also wish to express our thanks to the Schering Company for generous quantities of gentamicin complex fermentation mother liquors.

(18) Hoffmann LaRoche, Inc., Nutley, 10, N. J.

Hubert Maehr,[18] Carl P. Schaffner
Institute of Microbiology, Rutgers, The State University
New Brunswick, New Jersey 08903
Received September 21, 1967

(11) H. Maehr, Ph.D. Thesis, Rutgers University, New Brunswick, N. J., 1964.

(12) For the convention of numbering the atoms of 2-deoxystreptamine see K. L. Rinehart, Jr., M. Hichens, A. D. Argoudelis, W. S. Chilton, H. E. Carter, M. P. Georgiadis, C. P. Schaffner, and R. T. Schillings, *J. Am. Chem. Soc.*, **84**, 3218 (1962).

(13) (a) M. Hichens and K. L. Rinehart, Jr., *ibid.*, **85**, 1547 (1963). (b) S. Tatsuoka and S. Horii, *Proc. Japan Acad.*, **39**, 314 (1963).

(14) (a) G. Hagemann, G. Nomine, and L. Penasse, *Ann. Pharm. Franc.*, **16**, 585 (1958); (b) S. Horii, T. Yamaguchi, H. Hitomi, and A. Miyake, *Chem. Pharm. Bull.* (Tokyo), **9**, 340 (1961).

(15) M. Murase, *J. Antibiotics* (Tokyo), **A14**, 367 (1961).

(16) E. Stahl, Ed., "Duennschicht-Chromatographie," Springer-Verlag, Berlin, 1962, p 311.

(17) N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn, and K. Folkers, *J. Am. Chem. Soc.*, **68**, 2557 (1946).

Stereochemistry of the Photosensitized Hydration of Olefins

Sir:

We recently described a new photochemical addition reaction in which alcohols and water add to the double bond of certain cyclohexenes and cycloheptenes, in the presence of various photosensitizers, to give tertiary ethers and alcohols, respectively.[1] An imporant aspect

(1) (a) P. J. Kropp, *J. Am. Chem. Soc.*, 88, 4091 (1966); (b) J. A. Marshall and R. D. Carroll, *ibid.*, 88, 4092 (1966).