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.

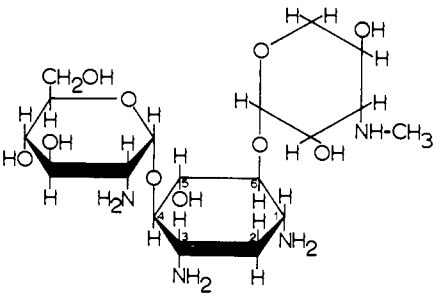
Methyl gentosaminide gives positive ninhydrin and Pan–Dutcher reactions; 1 mole reduced 2 moles of periodate accompanied by the liberation of 1 mole of methylamine. Methylamine was identified by tlc[16] and by its dinitrophenyl derivative, which did not depress the mixture melting point at 178° involving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

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(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.

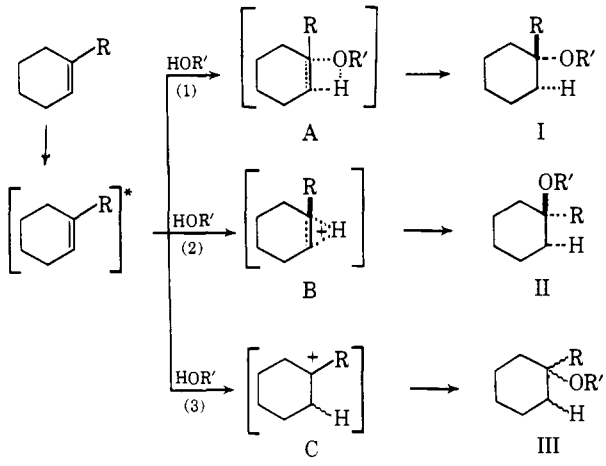
(17) N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn, and K. Folkers, *J. Am. Chem. Soc.*, **68**, 2557 (1946).

Stereochemistry of the Photosensitized Hydration of Olefins

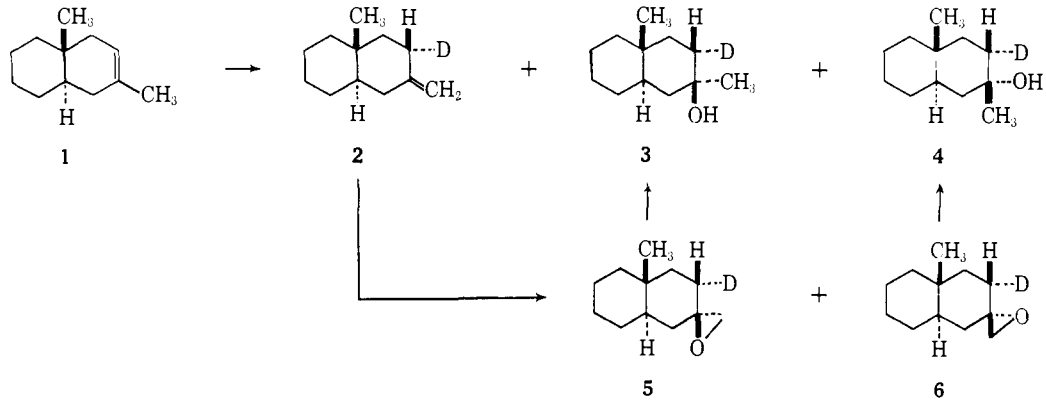
Sir:

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).



of this reaction which these early studies could not resolve concerns the stereochemistry of the addition process. *A priori*, three limiting cases may be envisioned: (1) a concerted four-center *cis* addition to the cyclo-



alkene (A → I),[2] (2) a *trans* addition, possibly *via* a protonated olefin,[2] and (3) a stepwise addition proceeding through a cationic species where product stereochemistry would be determined by the steric environment of the intermediate (C → III). We now report experimental evidence in favor of case 3.

The olefin employed for these studies, 2,10-dimethyl-*trans*-2-octalin (**1**), was prepared from *trans*-10-methyl-2-decalone[3] by addition of methyllithium and dehydration of the resulting alcohol mixture (2:1 axial–equatorial alcohol) with formic acid. Material thus secured contained less than 3% of olefin isomers as judged by gas chromatography and nmr analysis.

Irradiation[4] of olefin **1** in 1,2-dimethoxyethane–deuterium oxide containing xylene as the photosensitizer afforded the exocyclic isomer **2** [40% yield, 87% d_1; λ_{max}^{film} 3.28 (vinyl C–H), 4.59 (C–D), 6.06 (C=C), and 11.24 μ], the equatorial alcohol **3**[5] [30% yield, 89% d_1; λ_{max}^{film} 3.0 (OH), 4.60 (C–D), 7.73, 8.7, 9.35, 10.6, 10.8, and 11.5 μ], and the axial alcohol **4**[5] [20% yield, 89% d_1; λ_{max}^{film} 3.0 (OH), 4.60 (C–D), 8.50, 9.50, 9.96, 10.6, 11.4, and 13.0 μ] purified by preparative gas chromatography. The deuterated exocyclic olefin **2** afforded a mixture of oxiranes **5** and **6** (3:7)[6] upon treatment with *m*-chloroperoxybenzoic acid in methylene chloride. Reduction of this mixture with lithium aluminum hydride gave alcohols **3** and **4**, whose identity with the alcohols obtained directly from olefin **1** *via* the photochemical addition reaction was ascertained through the exact correspondence of their infrared and nmr spectra.

An authentic specimen of the deuterated alcohol **4** was prepared as follows. Olefin **1** was treated with diborane and oxidized successively with alkaline hydrogen peroxide and chromic acid to give *cis*-3,9-dimethyl-*trans*-2-decalone [**7**, λ_{max}^{film} 5.68 (CO), 8.20, 8.38, 8.52, 10.12, 10.70, 11.12 and 13.55 μ]. Reduction of ketone **7** with lithium aluminum deuteride afforded the alcohol **8** [λ_{max}^{film} 2.98 (OH), 4.75 (C–D), 9.5–9.6, 9.8, and 11.5 μ]. The methanesulfonate derivative **9** yielded the deuterated olefin **10** [97% d_1; λ_{max}^{film} 4.48 (C–D), 7.25, 15.0, and 15.6 μ] upon treatment with potassium *t*-butoxide in *t*-butyl alcohol. Epoxidation of this olefin and reduction of the epoxide **13** with lithium aluminum hydride afforded alcohol **4**. The identity of alcohol **4** synthesized in this manner with the axial alcohol obtained *via* photochemical addition of D_2O to olefin **1** was established by comparing their infrared and nmr spectra.

A sample of the axially deuterated axial alcohol **12** was prepared from olefin **1** by epoxidation and reduction of the resulting epoxide **11** with lithium aluminum deuteride. The infrared spectrum of this alcohol [λ_{max}^{film} 3.02 (OH), 4.60 (C–D), 8.21, 8.95, 9.05, 10.8, 11.1, and 12.7 μ] displayed numerous medium-to-strong bands not present in the spectrum of the equatorially deuterated axial alcohol **4**. These characteristic bands of alcohol **12** could be clearly detected in the spectrum of a 10:1 mixture of alcohols **4** and **12**. Since these bands were absent from the spectrum of the photochemically derived alcohol **4**, we conclude that the photochemical deuteration of olefin **1** occurs stereoselectively.

The aforementioned conversion of the exocyclic olefin **2** to a mixture of alcohols **3** and **4** and the independent synthesis of alcohol **4** outlined above establish an equatorial orientation for the deuterium at C-2 in the photochemical products **2**, **3**, and **4**. These findings suggest that protonation occurs from the less hindered face of the olefin giving an incipient cation. This species can then lose a proton to give an olefin or react

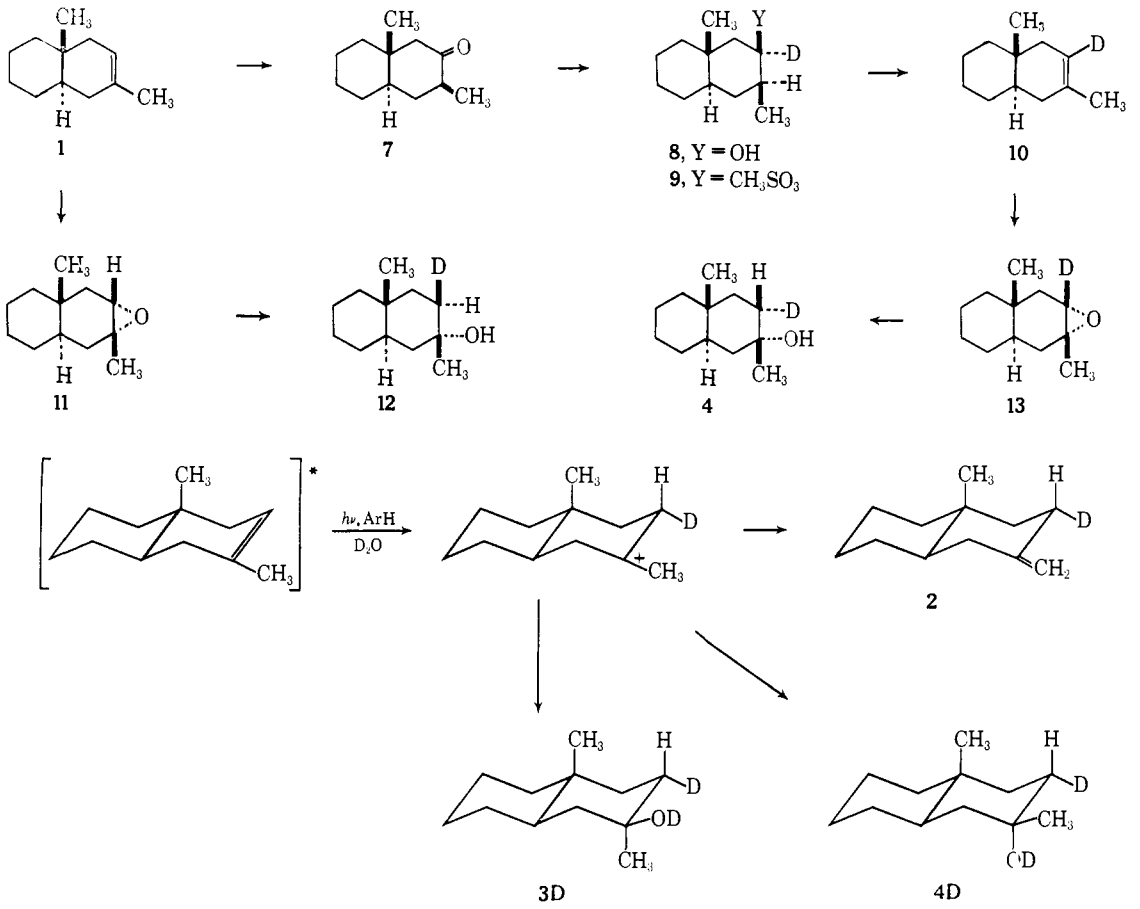
(2) A *trans*-cycloalkene has been suggested as the reactive species.[1a] In this event, *cis* addition could afford the *trans* addition product (A → II), and *vice versa* (B → I).

(3) *Cf.* J. A. Marshall, N. Cohen, and K. R. Arenson, *J. Org. Chem.*, **30**, 762 (1965), and references therein.

(4) A Hanovia 450-w high-pressure mercury vapor lamp (type L) was used with a water-jacketed Vycor immersion well.

(5) The products were washed with water to remove oxygen-bound deuterium.

(6) R. G. Carlson and N. S. Behn, *J. Org. Chem.*, **32**, 1363 (1967).



with water to produce alcohols **3** and **4**. In the latter case, steric factors favor formation of the equatorial alcohol **3**. Notably, samples of olefin **1** recovered from photochemical hydration experiments contained significant amounts (10–20%) of deuterium. Thus the proposed cation intermediate can collapse to endocyclic as well as to exocyclic olefin. Presumably both isomeric endocyclic olefins are thereby formed, but since we were unable to separate these olefins by gas chromatography we cannot yet confirm this point.

As noted previously, a certain amount of internal strain seems essential to the addition reaction.[1] Thus, exocyclic olefins such as **2** are unreactive. The hydration of olefin **1** proceeded about seven times as fast in H_2O as in D_2O. This rate difference underscores the importance of acidity [$K_A(H_2O)/K_A(D_2O) \sim 6$][7] to the addition reaction. Our previous findings on photochemically initiated alcohol additions[1b] and rearrangements[8] support this interpretation. The stereochemical results presented in this report clearly support a stepwise addition mechanism for photosensitized cycloalkene hydration and related reactions.[1] The nature of the species undergoing protonation is currently under investigation.

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(7) J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp 120–121.

(8) J. A. Marshall and A. R. Hochstetler, *Chem. Commun.*, 732 (1967).

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Steroids. CCCXXXIII.[1] Synthetic Studies on Insect Hormones. V.[2] The Synthesis of Crustecdysone (20-Hydroxyecdysone)

Sir:

The moulting hormone crustecdysone (20-hydroxyecdysone) (Ia) was first isolated from the crayfish *Jasus lalandei* and its structure proposed by Hampshire and Horn.[3] Later work showed this compound to be the major moulting hormone of the insect *Antherea pernyi*[4] and suggested the stereochemistry 20*R*,22*R* by analogy with that of the moulting hormone ecdysone[5]

(1) The material presented in this communication formed the basis of a presentation by one of us to a Gordon Research Conference on June 20, 1967. Part CCCXXXII: A. Cervantes, P. Crabbé, J. Iriarte, and G. Rosenkranz, submitted for publication.

(2) Part IV: J. B. Siddall, D. H. S. Horn, and E. J. Middleton, *Chem. Commun.*, 899 (1967).

(3) F. Hampshire and D. H. S. Horn, *ibid.*, 37 (1966). 20-Hydroxyecdysone has since been isolated from several plant and insect sources; *e.g.*, T. Takemoto, S. Ogawa, N. Nishimoto, and H. Hoffmeister, *Z. Naturforsch.*, **22b**, 681 (1967), and references therein.

(4) D. H. S. Horn, E. J. Middleton, J. A. Wunderlich, and F. Hampshire, *Chem. Commun.*, 339 (1966).

(5) 22*R* configuration determined by X-ray crystallography [R. Huber and W. Hoppe, *Chem. Ber.*, **98**, 2403 (1965)].