tive Pauly, Sakaguchi and Ehrlich positive spot with R_f[1] 0.61; single component on paper electrophoresis at pH 3.5; amino acid ratios in acid hydrolysate $ser_{2.17}tyr_{0.96}but_{1.13}glu_{1.00}his_{1.04}phe_{1.09}arg_{3.04}gly_{1.91}lys_{2.80}pro_{2.00}val_{1.91}$) was exposed to the action of anhydrous trifluoroacetic acid for 20 min. at room temperature to give crude I which was purified by chromatography on carboxymethylcellulose[22]; ($[\alpha]^{28}D$ −61.7° in 10% v./v. acetic acid; single ninhydrin, Pauly, Sakaguchi and Ehrlich positive spot with R_f[2] 0.7 × his; single component on disk electrophoresis at pH 4.6; amino acid ratios in acid hydrolysate $ser_{1.97}tyr_{0.91}but_{0.94}glu_{1.02}his_{1.02}phe_{0.98}arg_{2.91}gly_{1.89}lys_{3.31}pro_{2.16}val_{1.89}$). The observation that leucine aminopeptidase digests of I contain glutamic acid and not glutamine[23] demonstrates that the glutamine amide group is susceptible to hydrolysis by trifluoroacetic acid. Schwyzer, *et al.*,[24] exposed N-*t*-butyloxycarbonylseryltyrosylserylmethionylglutaminylhistidylphenylalanylarginyltryptophylglycyl - N^{ϵ} - *t* - butyloxycarbonyllysylprolylvalylglycyl - N^{ϵ} - *t* - butyloxycarbonyllysyl - N^{ϵ}-*t*-butyloxycarbonyllysylarginylarginylproline-*t*-butyl ester to the action of trifluoroacetic acid and stated, without experimental support, that this treatment did not remove the amide group from the glutamine residue. Our own results indicate that the glutamine amide group in sequences related to the N-terminus of the corticotropins undergoes significant hydrolysis on exposure to trifluoroacetic acid.

Acknowledgment.—The skillful technical assistance of Mrs. Chizuko Yanaihara, Mrs. Maria Gunther, Miss Priscilla Holland and Mr. John Humes is gratefully acknowledged.

(22) E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751 (1956).

(23) The enzyme preparation used in these studies fails to show glutaminase activity.

(24) R. Schwyzer, W. Rittel, H. Kappeler and B. Iselin, *Angew. Chem.*, **72**, 915 (1960).

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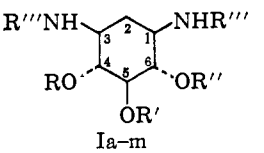
RECEIVED MARCH 7, 1963

CHEMISTRY OF THE NEOMYCINS. XII[1] THE ABSOLUTE CONFIGURATION OF DEOXYSTREPTAMINE IN THE NEOMYCINS, PAROMOMYCINS AND KANAMYCINS

Sir:

The final uncertainty in the stereochemistry[1] of neomycin C, the absolute configuration of the unsymmetrically substituted deoxystreptamine portion of the antibiotic, now has been resolved.

N,N′-Diacetyl-6-O-methyldeoxystreptamine (Ib),[2-4] m.p. 280–284° dec., $[\alpha]^{28}D$ +5.0° (*c* 0.64, water), was prepared from poly-O-methyl-hexa-N-acetyl-neomycin B. This compound has been isolated previously from neomycin B (reported m.p. 282–284° dec., $[\alpha]^{27}D$ +12°),[2] from paromomycin (reported m.p. 280–282°, $[\alpha]^{27}D$ +15°),[3a] and from zygomycins A_1 and A_2 (reported m.p. 280–283°, $[\alpha]^{24}D$ +4°),[3b] which from their properties and degradation products are probably identical with paromomycin and paromomycin II,[2] respectively.



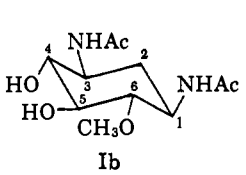
Ia–m

Ia (Deoxystreptamine): R = R′ = R″ = R‴ = H
Ib: R = R′ = H; R″ = CH_3; R‴ = $COCH_3$
Ic: R = CH_3; R′ = R″ = H; R‴ = $COCH_3$
Id: R = H; R′ = R″ = CH_3; R‴ = $COCH_3$

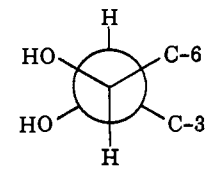
Ie (Neamine):	R = Neosamine C; R′ = R″ = R‴ = H
If (Neomycin C):	R = Neosamine C; R′ = Neobiosamine C; R″ = R‴ = H
Ig (Neomycin B):	R = Neosamine C; R′ = Neobiosamine B; R″ = R‴ = H
Ih (Paromomycin):	R = D-Glucosamine; R′ = Neobiosamine B; R″ = R‴ = H
Ii (Paromomycin II): (suggested)	R = D-Glucosamine; R′ = Neobiosamine C; R″ = R‴ = H
Ij (Paromamine):	R = D-Glucosamine; R = R″ = R‴ = H
Ik (Kanamycin C):	R = D-Glucosamine; R′ = R‴ = H; R″ = Kanosamine
Il (Kanamycin A):	R = 6-Aminoglucose; R′ = R‴ = H; R″ = Kanosamine
Im (Kanamycin B):	R = Diaminohexose; R′ = R‴ = H; R″ = Kanosamine

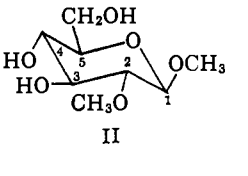
The work of Reeves[5] provides a tool for the determination of the stereochemical relationship between the remaining adjacent hydroxyl groups of Ib, by measuring the change in optical rotation, $\Delta[M]_{\text{Cupra B}}$, when cuprammonium hydroxide solution ("Cupra B") is substituted for water as the solvent.

The above compound (Ib) has $[\alpha]^{27}_{436}$ +9.8° (*c* 0.6, H_2O) and $[\alpha]^{27}_{436}$ +620° (*c* 0.47, Cupra B), giving $\Delta[M]_{\text{Cupra B}}$ = +1590. The high positive increment is similar to that observed with methyl 2-O-methyl-β-D-glucoside, II ($\Delta[M]_{\text{Cupra B}}$ = +2190) but of opposite sign to that of methyl 4-O-methyl-β-D-glucoside, III ($\Delta[M]_{\text{Cupra B}}$ = −2020).[6] Thus, the adjacent hydroxyl groups in 6-O-methyldeoxystreptamine are related as those at C-3 and C-4 of the glucopyranose ring,[7] *i.e.*, 6-O-methyldeoxystreptamine has structure Ib, rather than that of its mirror image Ic.

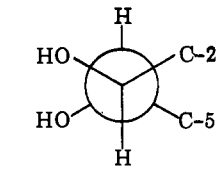


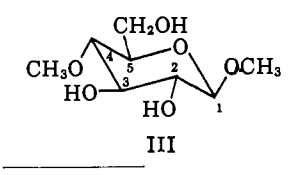
Ib



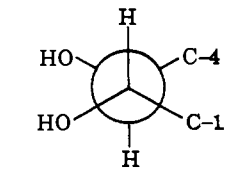


II





III



(1) Paper XI in this series: K. L. Rinehart, Jr., W. S. Chilton, M. Hichens and W. v. Phillipsborn, *J. Am. Chem. Soc.*, **84**, 3216 (1962).

(2) K. L. Rinehart, Jr., M. Hichens, A. D. Argoudelis, W. S. Chilton, H. E. Carter, M. Georgiadis, C. P. Schaffner and R. T. Schillings, *ibid.*, **84**, 3218 (1962).

(3) (a) T. H. Haskell, J. C. French and Q. R. Bartz, *ibid.*, **81**, 3483 (1959); (b) S. Horii, *J. Antibiotics* (Tokyo), *Ser. A*, **15**, 187 (1962).

(4) The numbering shown is that defined recently[2] for deoxystreptamine in neamine in order to place neosamine C on the lower numbered carbon. It is seen in the present report to have the additional advantage that it gives the lower number to the hydroxyl-bearing carbon (C-4) of absolute stereochemistry *R*.

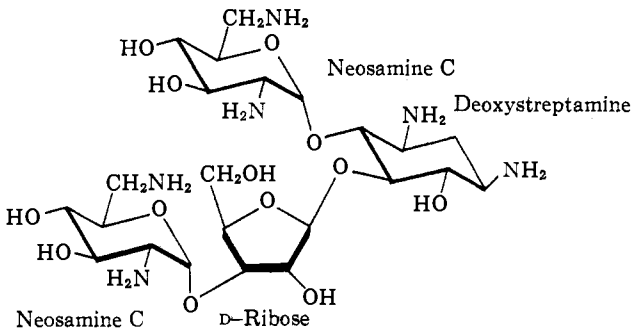
(5) R. E. Reeves, *Advan. Carbohydrate Chem.*, **6**, 107 (1951).

(6) $\Delta[M]_{\text{Cupra B}} = ([\alpha]_{436}\text{ Cupra B} - [\alpha]_{436}\text{ water}) \times \frac{\text{mol. wt.}}{100}$.

(7) The possibility of unexpected interference by the acetamido groups in the cuprammonium complex is eliminated by the observation that crystalline N,N,-diacetyl-5,6-di-O-methyldeoxystreptamine (Id, sublimes above 260° without melting; *Anal.* Found: C, 52.70; H, 8.24; N, 10.68; OCH_3, 22.6), prepared from poly-O-methyl-tetra-N-acetylneamine, exhibited no enhanced rotation in Cupra B solution.

From this assignment, it follows directly that the absolute stereochemistry of deoxystreptamine in neamine is as shown in Ie. The neamine component of neomycins B and C is identical and substituted deoxystreptamine structures If and Ig may be written for the antibiotics themselves. Since Ib is also obtained from poly-O-methyl-penta-N-acetylparomomycin,[3a] and from the poly-O-methyl-N-acetyl derivatives of zygomycins A_1 and A_2[3b] (probably identical with paromomycins I and II), the substituted deoxystreptamines in these antibiotics are Ih for paromomycin–zygomycin A_1, and probably Ii for paromomycin II–zygomycin A_2. Moreover, the very recent isolation of paromamine[8] [Ij; 4-(2-amino-2-deoxy-α-D-glucosyl)-deoxystreptamine] from partial hydrolysis of kanamycin C[9] establishes the absolute configuration of deoxystreptamine in that antibiotic[10] as in Ik. Also, since kanamycins A[11] and B,[12] like kanamycin C,[10] contain kanosamine (3-amino-3-deoxy-D-glucose) and deoxystreptamine, but differ from kanamycin C in the replacement of its glucosamine fragment by 6-aminoglucose in kanamycin A and by an unidentified diaminohexose[13] (on biogenetic grounds this should be neosamine C) in kanamycin B, the absolute stereochemistry of deoxystreptamine in these antibiotics is almost certainly as in Il and Im, respectively.

With the assignment of this last stereochemical point the structure of neomycin C is completed and may be written as shown.



Neomycin C

Complete structures of neomycin B and paromomycin await further evidence for the stereochemistry of neosamine B. The cuprammonium method provides further evidence on this point, as well. As expected, a high positive increment is observed in the rotation of methyl N,N′-diacetyl-α-neosaminide C (a 2,6-diamino-D-glucose derivative, with free hydroxyl groups at C-3 and C-4) in cuprammonium B solution; $\Delta[M]_{\text{Cupra B}} = +2280°$. Of greater significance is the strong positive increment of methyl N,N′-diacetyl-α-neosaminide B; $\Delta[M]_{\text{Cupra B}} = +1880°$. Though this observation does not of itself exclude L-talose or L-altrose stereochemistry for neosamine B, it does exclude the five other isomers allowed by neosamine B's stereochemistry at C-2[14,15] (with the amino group on the right in the Fischer projection formula) and is most consistent with the previously suggested[2] 2,6-diamino-2,6-dideoxy-L-idose stereochemistry.

(8) T. H. Haskell, J. C. French and Q. R. Bartz, *J. Am. Chem. Soc.*, **81**, 3480 (1959).

(9) T. Wakazawa and S. Fukatsu, *J. Antibiotics* (Tokyo), *Ser. A*, **15**, 225 (1962).

(10) M. Murase, *ibid.*, **14**, 367 (1961).

(11) M. J. Cron, D. L. Evans, F. M. Palermiti, D. F. Whitehead, I. R. Hooper, P. Chu and R. U. Lemieux, *J. Am. Chem. Soc.*, **80**, 4742 (1958).

(12) H. Schmitz, O. B. Fardig, F. A. O'Herron, M. A. Rousche and I. R. Hooper, *ibid.*, **80**, 2912 (1958).

(13) I. R. Hooper, personal communication.

(14) K. L. Rinehart, Jr., A. D. Argoudelis, T. P. Culbertson, W. S. Chilton and K. Striegler, *J. Am. Chem. Soc.*, **82**, 2979 (1960).

(15) T. H. Haskell, J. C. French and Q. R. Bartz, *ibid.*, **81**, 3481 (1959).

Acknowledgments.—This investigation was supported in part by research grant No. E-1278 from the National Institute of Allergy and Infectious Diseases, Public Health Service. We also wish to express our thanks to the Upjohn Company for neomycin samples, to Mr. W. S. Chilton for assistance in carrying out some of the experiments and, particularly, to Professor Gabor Fodor, who, during discussions in Budapest, raised the possibility of application of Reeves' method to this problem.

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1,1,1,4,4,4-HEXAFLUORO-2,3-DIPHENYL-2,3-BUTANEDIOL

Sir:

We wish to report the observation of a remarkable resistance to pinacol–pinacolone rearrangement exhibited by a symmetrical alkyl aryl ethanediol.

1,1,1,4,4,4-Hexafluoro-2,3-diphenyl-2,3-butanediol (I) was prepared in 17% yield by the photopinacolization reaction between 13 g. of trifluoromethyl phenyl ketone[1] and 75 ml. of 2-propanol in a quartz tube at 5 cm. from a Hanovia 16A-13 mercury vapor lamp. Distillation of the solvent and recrystallization of the residue from 80:20 hexane–ether produced 2.16 g. of white needles. These were further purified by sublimation *in vacuo;* m.p. 155–156°. (*Anal.* Calcd. for $C_{16}H_{12}O_2F_6$: C, 54.86; H, 3.45. Found: C, 55.10; H, 3.38.)

The compound possessed an infrared spectrum, in KBr disk, completely consistent with its assignment as the pinacol: —OH, 3440–3600 cm.$^{-1}$; —CF_3,[2] 1165–1210 cm.$^{-1}$; and the complete absence of carbonyl absorptions.

Oxidative cleavage of I with lead tetraacetate in boiling acetic acid gave only trifluoromethyl phenyl ketone, identified as its 2,4-dinitrophenylhydrazone: m.p. and mixture m.p. 108–109°; literature m.p. 106–107°.[3]

The pinacol was found to be completely resistant to several concentrations of sulfuric acid in acetic acid at steam bath temperatures. It could be recovered unchanged from 38% sulfuric acid after 4 hr. heating and from 65% sulfuric after 6 hr. heating. It resisted the action of boiling acetic acid-β-naphthalenesulfonic acid for 30 hr.

The non-fluorinated analog of I, 2,3-diphenyl-2,3-butanediol,[4] prepared by the addition of excess methyllithium to benzil, could be transformed into 3,3-diphenyl-2-butanone by much less vigorous conditions: 0.6% *p*-toluenesulfonic acid or 0.6% iodine in acetic acid after 0.5 hr. reflux.

The resistance of I to acidic conditions substantially in excess of those required to rearrange similar diols may be interpreted as a destabilizing influence of the powerfully electron attracting trifluoromethyl group to the development of carbonium character on the hydroxyl-bearing carbons. Such carbonium character is generally agreed to be a prerequisite to the rearrangement step.[5]

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RECEIVED MARCH 21, 1963

(1) The ketone was synthesized according to the procedure of A. Sykes, J. C. Tatlow and C. R. Thomas, *Chem. Ind.* (London), 630 (1955).

(2) In agreement with previous spectral investigations of the —CF_3 group, an intense absorption band consisting of a series of multiple peaks is observed: L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd. Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, p. 330.

(3) A. Sykes, J. C. Tatlow and C. R. Thomas, *J. Chem. Soc.*, 835 (1956).

(4) D. Cram and K. Kopecky, *J. Am. Chem. Soc.*, **81**, 2748 (1959).

(5) J. F. Duncan and K. R. Lynn, *Australian J. Chem.*, **10**, 7 (1957).

(6) N.S.F. Coöperative Graduate Fellow, 1959–63.