

However, if the axial orientation were preferred, 2 (the diaxial chair conformation of *trans*-I) would be more stable than 5. In either case, the chair conformation of *cis*-I (5), with one axial and one equatorial *t*-butyl group, would be expected to be intermediate in stability between 1 and 2. If this reasoning is valid, then because *cis*-I proved to be more stable than *trans*-I (Table I), *cis*-I must be able to exist in non-chair conformations which are more stable than 1, 2 or 5. Possible non-chair conformations for *cis*-I include 6, 7 and 8.

Allinger⁸ has reported calculations which suggest that the chair, boat and twist conformations of 1,4-cyclohexanedione (2, 3 and 4 with R = H) have comparable energies (perhaps within ± 0.2 kcal./mole). Assuming such to be the case, one can estimate the relative energy change resulting from substitution of a *t*-butyl group at each position of each of these conformations. The *t*-butyl groups appear to be most comfortably positioned in 7 for *cis*-I, and in 3 for *trans*-I, but with 7 somewhat more favorable than 3.⁹ Therefore, like *cis*-I, *trans*-I may prefer a non-chair conformation (such as 3). The equilibration results are not inconsistent with this possibility, since *cis*-I and *trans*-I were found to have comparable entropies² (Table I).

The equilibration results clearly demonstrate that the diequatorial chair conformation of *trans*-2,5-di-*t*-butyl-1,4-cyclohexanedione (1) does not enjoy the special stability possessed by the diequatorial chair conformations of the related cyclohexane¹ and cyclohexanone² derivatives. The results are in accord with the description of 1,4-cyclohexanedione proposed by Allinger.⁸ We are now exploring the possibility that non-chair conformations may also predominate for other simple 1,4-cyclohexanediones.

We are indebted to Dr. J. Casanova, Jr., for assistance with apparatus for gas chromatography. We wish to express our appreciation of support by the Research Corporation.

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(9) The boat 7 appears more stable than 6 because in 7 each carbonyl oxygen is skew (rather than opposed) to the adjacent *t*-butyl group. However, the most stable conformation of *cis*-I may be a non-chair conformation intermediate between 7 and 8. Note that twist conformation 4 of *trans*-I is destabilized by a 1,4-repulsion between one *t*-butyl group and one hydrogen which can be relieved by rotation toward 3.

CONTRIBUTION No. 272

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THE STRUCTURE OF NEAMINE

Sir:

On methanolysis in 0.4 *N* hydrochloric acid¹ neomycins B and C are approximately bisected to give the methyl glycosides of neobiosamines B and C, respectively,¹ together with the fragment neamine,^{1,2,3,4} common to both. The structures of

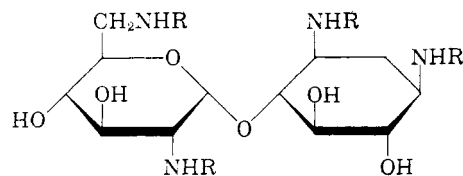
(1) J. D. Dutcher, N. Hosansky, M. N. Donin and O. Wintersteiner, *J. Am. Chem. Soc.*, **73**, 1384 (1951).

(2) R. L. Peck, C. E. Hoffhine, Jr., P. Gale and K. Folkers, *ibid.*, **71**, 2590 (1949); **75**, 1018 (1953).

(3) B. E. Leach and C. M. Teeters, *ibid.*, **73**, 2794 (1951); **74**, 3187 (1952).

(4) J. D. Dutcher and M. N. Donin, *ibid.*, **74**, 3420 (1952).

neobiosamines B⁵ and C⁶ have been assigned previously; the present report establishes the structure of neamine as I.

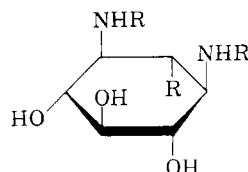


Neosamine C Deoxystreptamine

I, R = H (Neamine)

II, R = COCH₃

III, R = COC₆H₅



IV, R = H, R' = H

V, R = COC₆H₅, R' = H

VI, R = H, R' = OH

VII, R = COC₆H₅, R' = OH
(R' inside ring)

Neamine (C₁₂H₂₆N₄O₆)² is hydrolyzed completely during 9 hours by refluxing 48% hydrobromic acid to give an 83% yield of deoxystreptamine (IV, C₆H₁₄N₂O₃),⁷ whose gross 1,3-diamino-4,5,6-trihydroxycyclohexane structure was established by Kuehl, Bishop and Folkers from degradative evidence.⁷ The all-*trans* stereochemistry of IV is assigned in the present study from these observations: (1) a *trans* OH-NH₂ relationship is indicated by the failure of N,N'-dibenzoyldeoxystreptamine (V),⁷ m.p. 312–313°, to undergo N → O benzoyl migration under conditions (0.7 *N* hydrochloric acid in 95% ethanol, room temperature, two weeks) where *cis*-2-benzamidocyclohexanol undergoes N → O benzoyl migration, but the *trans* isomer does not.⁸ Significantly, N,N'-dibenzoylstreptamine (VII),⁹ m.p. 287–288°, which has been assigned the all-*trans* configuration on synthetic evidence,¹⁰ also fails under the present conditions to undergo N → O benzoyl migration. (2) A *trans* OH-OH configuration is argued by the nearly equal rates of reaction with 0.1 *N* periodate of V (which is actually slightly slower) and the streptamine analog (VII).

Neamine was acetylated in the present study by acetic anhydride in methanol at 0–5° to the known² N,N',N'',N'''-tetraacetyneamine (II). Hydrolysis of II in 3 *N* aqueous hydrochloric acid during 10 hours on a steam-bath gave a mixture of organic bases. Cellulose chromatography¹¹ (BAW 221 solvent system)¹² of the hydrolyzate from 975

(5) K. L. Rinehart, Jr., A. D. Argoudelis, T. P. Culbertson, W. S. Chilton and K. Striegler, *ibid.*, **82**, 2970 (1960).

(6) K. L. Rinehart, Jr., and P. W. K. Woo, *ibid.*, **80**, 6463 (1958).

(7) F. A. Kuehl, Jr., M. N. Bishop and K. Folkers, *ibid.*, **73**, 881 (1951). These authors, who established a *meso* configuration by optical inactivity, suggested the all-*trans* configuration for IV on the compound's presumed biogenetic relation to streptamine (VI).

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(9) H. E. Carter, Y. H. Loo and J. W. Rothrock, *J. Biol. Chem.*, **179**, 1027 (1949).

(10) M. L. Wolfrom, S. M. Olin and W. J. Polglase, *J. Am. Chem. Soc.*, **72**, 1724 (1950).

(11) Purification was also effected by gradient elution with hydrochloric acid from a Dowex 50 (Dow Chemical Co. strongly acidic cation exchange resin) ion exchange column.

(12) K. L. Rinehart, Jr., A. D. Argoudelis, W. A. Goss, A. Sohler and C. P. Schaffner, *J. Am. Chem. Soc.*, **82**, 3938 (1960).

mg. of II gave these fractions: (1), 86 mg. of neosamine C, dihydrochloride, $[\alpha]^{25}_D +65^\circ$ (c 1.0, water) [lit.¹³ $[\alpha]^{25}_D +69^\circ$ (c 0.87, water)], R_f (BAW 221) 0.17 (lit.¹² 0.17); (2), an intermediate fraction, whose components were identified by papergrams and ion exchange,¹¹ and which was calculated from rotation to contain 145 mg. of neosamine C and 72 mg. of deoxystreptamine hydrochloride; (3), 160 mg. of optically inactive deoxystreptamine hydrochloride; (4), 430 mg. of recovered neamine, hydrochloride $[\alpha]^{25}_D +82^\circ$ (c 1.0, water) [lit.² $[\alpha]^{25}_D +83^\circ$ (c 1.0, water)].

Conclusive evidence for the identity of the hydrolysis product and neosamine C was provided by the N-acetylation¹⁴ of the former to N,N'-diacetylneosamine C, m.p. 209–221° (undepressed on mixture with an authentic sample¹⁵ melting 210–214°), $[\alpha]^{25}_D +38^\circ$ (c 0.6, water) [lit.¹⁵ $[\alpha]^{25}_D +37^\circ$ (c 0.8, water), R_{NAG} (BAW 415) 1.35 (lit.¹⁵ 1.32). Neosamine was earlier assigned D-glucose stereochemistry¹⁶ from degradative and rotation evidence; the assignment has been confirmed recently by comparison of the diaminoheptose with synthetic 2,6-diamino-2,6-dideoxy-D-glucose.¹⁵

Both N,N',N'',N'''-tetraacetylneamine (II) and N,N',N'',N'''-tetrabenzoylneamine (III) consume selectively two moles of periodate when treated at room temperature with 0.1 *N* periodate. Since a maximum of one mole of periodate may be consumed in *vic*-glycol cleavage of the deoxystreptamine moiety and a like amount in the neosamine C moiety in II and III, these results establish a 4- (rather than 5-) glycoside linkage on deoxystreptamine and a pyranose (rather than furanose) structure for neosamine C in neamine. Confirmation of the attachment of neosamine C at C-4 in deoxystreptamine (providing a *vic* glycol for cleavage) is provided by the observation that no deoxystreptamine may be detected after the two-molar periodate oxidation of II, with subsequent hydrolysis with refluxing 48% hydrobromic acid (*cf.* above).

From the rotation of methyl N,N'-diacetyl- α -neosaminide C,¹⁵ $[M]_D +32,800$, and the value of A_{Ac} for methyl N-acetyl-D-glucosaminides,¹⁷ +17,390, one may derive by Hudson's rules¹⁸ the value $B_{AcC} +15,400$ for N,N'-diacetylneosaminides C, while the rotation of a 4-substituted N,N'-diacetyldeoxystreptamine is known from $[M]_D +3,900$ for 4-O-methyl-N,N'-diacetyldeoxystreptamine.¹⁹ Combination of these values and the molecular rotation of N-tetraacetylneamine (II) into the equation $[M]_{AcNe} = [M]_{AcDe} + A_{AcC}$

+ B_{AcC} , where $[M]_{AcNe} +43,300$ is the molecular rotation of II, $[M]_{AcDe}$ is the rotational contribution of the 4-substituted-N,N'-diacetyldeoxystreptamine moiety, and A_{AcC} and B_{AcC} have the usual meanings¹⁸ for a glycoside of N,N'-diacetylneamine, C, yields $A_{AcC} = +24,000$ or $+31,800$ (depending on the sign of $[M]_{AcDe}$).²⁰ In either case the high positive value agrees well with that expected for an α -linked glycoside (as shown in I).^{18,20} This conclusion may also be reached, though less quantitatively, from the high molecular rotation of neamine hydrochloride ($[M]_D +38,800$)² relative to the corresponding value for neosamine C hydrochloride ($[M]_D +16,800$).¹⁶ Except for the assignment of absolute stereochemistry to the substituted deoxystreptamine (*i.e.*, 4- vs. 6-substitution), the present data establish the structure for neamine.

Acknowledgment.—This investigation was supported in part by research grants, No. E-618 and No. E-1278, from the National Institute of Allergy and Infectious Diseases, Public Health Service, and by a Fellowship from the Upjohn Company. We also wish to express our thanks to the Upjohn Company for generous quantities of neomycin samples.

(20) Recent work elsewhere (Dr. C. P. Schaffner, personal communication) has shown that the 4-O-methyl-N,N'-diacetyldeoxystreptamine obtained by N-acetylation, O-methylation, and hydrolysis of neomycin B has the same rotation (positive) as the same compound from the analogous sequence on paromomycin.¹⁹ Hence, $[M]_{AcDe}$ is $-3,900$ and A_{AcC} is $+31,800$.

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A STUDY OF THE REACTIONS OF VARIOUS TIN(II) COMPOUNDS WITH CALCIUM HYDROXYLAPATITE

Sir:

We wish to report the results of a series of investigations carried out in aqueous media involving the reaction between calcium hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$, and these tin(II) salts: SnF_2 , $SnCl_2 \cdot 2H_2O$, $SnSO_4$, $SnClF$, and Sn_2ClF_3 . The reactions led in each case, except for that involving $SnCl_2 \cdot 2H_2O$, to the formation of a basic tin(II) phosphate having a molar Sn/PO_4 ratio of 2.0, to which we have assigned the empirical formula $Sn_4(PO_4)_2(OH)_2 \cdot H_2O$ (calculated: Sn, 66.2; PO_4 , 26.5. Found: Sn, 65.8; PO_4 , 26.5). The reaction involving $SnCl_2 \cdot 2H_2O$, however, resulted in the formation of $Sn_3(PO_4)_2$.

Experimental.—Calcium hydroxylapatite for use in these studies was prepared according to the method of Hayek and Stadlmann.¹ Stannous fluoride was obtained by treating SnO with HF .² The mixed chlorofluorides were prepared according to procedures previously outlined in this laboratory.^{3,4} Commercial preparations of both $SnCl_2 \cdot 2H_2O$ and $SnSO_4$ were employed.

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