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**.

Allinger[8] 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 entropies[2] (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 cyclohexane[1] and cyclohexanone[2] 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.

(8) N. L. Allinger, *J. Am. Chem. Soc.*, **81**, 5727 (1959).

(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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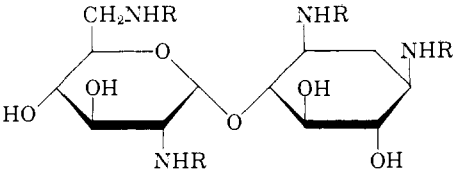
THE STRUCTURE OF NEAMINE

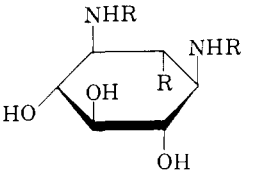
Sir:

On methanolysis in 0.4 *N* hydrochloric acid[1] 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 neobiosamines B[5] and C[6] have been assigned previously; the present report establishes the structure of neamine as I.



Neosamine C Deoxystreptamine

I, R = H (Neamine)
II, R = $COCH_3$
III, R = COC_6H_5



IV, R = H, R′ = H
V, R = COC_6H_5, R′ = H
VI, R = H, R′ = OH
VII, R = COC_6H_5, R′ = OH
(R′ inside ring)

Neamine ($C_{12}H_{26}N_4O_6$)[2] is hydrolyzed completely during 9 hours by refluxing 48% hydrobromic acid to give an 83% yield of deoxystreptamine (IV, $C_6H_{14}N_2O_3$),[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–NH_2 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 known[2] N,N′,N″,N‴-tetraacetylneamine (II). Hydrolysis of II in 3 *N* aqueous hydrochloric acid during 10 hours on a steam-bath gave a mixture of organic bases. Cellulose chromatography[11] (BAW 221 solvent system)[12] of the hydrolyzate from 975

(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).

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(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).

(8) G. Fodor and J. Kiss, *Acta Chim. Acad. Sci. Hung.*, **1**, 130 (1951).

(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, $[\alpha]^{28}D$ +65° (*c* 1.0, water) [lit.[13] $[\alpha]^{23}D$ +69° (*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 $[\alpha]^{28}D$ +82° (*c* 1.0, water) [lit.[2] $[\alpha]^{25}D$ +83° (*c* 1.0, water).

Conclusive evidence for the identity of the hydrolysis product and neosamine C was provided by the N-acetylation[14] of the former to N,N′-diacetylneosamine C, m.p. 209–221° (undepressed on mixture with an authentic sample[15] melting 210–214°), $[\alpha]^{28}D$ +38° (*c*, 0.6, water) [lit.[15] $[\alpha]^{25}D$ +37° (*c* 0.8, water), R_{NAG} (BAW 415) 1.35 (lit.[15] 1.32). Neosamine was earlier assigned D-glucose stereochemistry[16] from degradative and rotation evidence; the assignment has been confirmed recently by comparison of the diaminohexose 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_G for methyl N-acetyl-D-glucosaminides,[17] +17,390, one may derive by Hudson's rules[18] 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 meanings[18] for a glycoside of N,N′-diacetylneosamine, 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.

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(18) C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909); *cf.* W. Pigman, in "The Carbohydrates," Academic Press, Inc., New York, N. Y., 1957, p. 70.

(19) T. H. Haskell, J. C. French and Q. R. Bartz, *J. Am. Chem. Soc.* **81**, 3482 (1959).

(20) Recent work elsewhere (Dr. C. P. Schaffner, personal communication) has showed 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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