Medical Research, contained a small amount of streptomycin impurity. The dihydro derivatives were prepared from these samples by catalytic reduction.

A solution of 5 μg. of streptomycin trihydrochloride or hydroxystreptomycin trihydrochloride (25 μg. of the mannosido compounds) in 0.005 cc. of water was applied with a micropipet to a strip of Whatman No. 4 paper 0.5″ by 17.5″. The developing solvent was wet butanol containing 2% *p*-toluenesulfonic acid and 2% piperidine. After development the solvent was removed by washing the paper with ether and drying at room temperature. The strip was then placed on an agar layer inoculated with *Staphylococcus aureus*. Ten minutes was allowed for the streptomycin to diffuse into the agar. The strips were then removed and the trays incubated overnight at 37°.

PEORIA 5, ILLINOIS RECEIVED DECEMBER 1, 1950

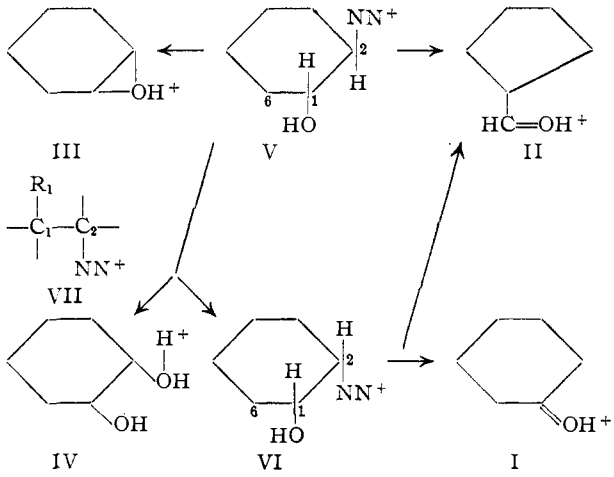
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TORONTO]

Pinacolic Rearrangements of Epimeric Aminocyclanols[1]

BY G. E. MCCASLAND

cis-2-Aminocyclohexanol on treatment with nitrous acid undergoes a pinacol rearrangement, yielding both cyclohexanone and cyclopentylmethanal. The *trans* epimer yields almost exclusively cyclopentylmethanal. Possible mechanisms are discussed in relation to configuration and conformation. Aminocyclanols often give anomalous high Van Slyke amino nitrogen values, which cannot be accounted for by reaction of an intermediate carbonyl compound, diol or epoxide with excess nitrous acid.

The formation of a ketone (I), aldehyde (II), epoxide (III) or diol (IV) from a 2-aminoalkanol by reaction with nitrous acid is conveniently explained in terms of the displacement of N_2 from the presumed diazonium intermediate (V or VI) by a migrating or entering electron-rich group.[2] The products I–IV are shown in protonated form. Ketone and aldehyde result from the migration of H: and R: respectively. Epoxide may result from internal displacement of N_2 by the carbinol oxygen, and diol by external displacement of N_2 by a molecule of the solvent (water).



Influence of Configuration and Conformation.— Pollak and Curtin[3] have recently discussed the effect of configuration on migrations in diastereomeric 2-aminoalkanols. As they suggest, a 1–2 shift of R_1 should be favored by that conformation of the molecule in which R_1 is *trans and coplanar* with respect to the diazonium ion group and carbon atoms 1 and 2 (formula VII). The fraction of molecules having this favorable *conformation* is in turn influenced by the *configuration*.

The application of this hypothesis to the 2-aminocyclohexanols requires a consideration of the equatorial and polar conformations[4] of the cyclohexane ring. Here the ring itself at carbon 6 constitutes one migrating group, and the hydrogen atom at carbon 1 constitutes the other.

In the case of *trans*-2-aminocyclohexanol, hydrogen 1 is *cis* to the diazonium ion group (V). Such 1,2-*cis* cyclohexane derivatives exist as a non-resolvable mixture of two antimeric equatorial-polar (*e*,*p*) conformations. When the diazonium ion group is *p*, hydrogen 1 must be *e*, and *vice versa*. Examination of models shows that when the diazonium group has the *e* position, ring carbon 6 is in the favored (*i.e. trans* coplanar) position for migration, but hydrogen 1 is not. When the diazonium group has the *p*-position, *neither* ring carbon 6 nor hydrogen 1 is in a favorable position for migration.

In *cis*-2-aminocyclohexanol, however, hydrogen 1 is *trans* to the diazonium group. Therefore, either hydrogen 1 and the diazonium ion group are both polar (*p*,*p*), or both are equatorial (*e*,*e*). Examination of models reveals that the (*p*,*p*) conformation favors migration of hydrogen 1, while the (*e*,*e*) favors migration of ring carbon 6. The relative extent of each migration might then depend on the relative proportion of (*p*,*p*) and (*e*,*e*) molecules.

On this basis it would be predicted that the carbonyl product formed by rearrangement of *trans*-2-aminocyclohexanol would consist exclusively of the aldehyde II (ring-contraction); while the *cis* epimer would yield a mixture containing both this aldehyde and the uncontracted ketone (I).

Experimentally, treatment of *trans*-2-aminocyclohexanol with nitrous acid gave a high yield of cyclopentylmethanal, confirming a similar finding by

(1) For related publications see: (a) G. E. McCasland, THIS JOURNAL, **73**, 2295 (1951); (b) McCasland and Smith, *ibid.*, **73**, 2190 (1950); (c) McCasland, Clark and Carter, *ibid.*, **71**, 637 (1949); (d) Carter, Clark, Lytle and McCasland, *J. Biol. Chem.*, **175**, 683 (1948); (e) Carter, Belinskey, Clark, Flynn, Lytle, McCasland and Robbins, *ibid.*, **174**, 415 (1948).

(2) The reaction of acyclic amino-alcohols has been extensively studied, in particular by McKenzie and co-workers, *e.g.*, *Ber.*, **63**, 904 (1930). Whitmore played an important role in formulating the mechanism here favored (*e.g.*, see THIS JOURNAL, **61**, 1324 (1939)). For further discussion and citation of the numerous references see Chap. 12 in G. W. Wheland, "Advanced Organic Chemistry," (John Wiley and Sons, Inc., New York, N. Y., 1949) and Chap. 12 by E. S. Wallis in Gilman's "Advanced Treatise," John Wiley and Sons, Inc., New York, N. Y., 2nd ed., 1944.

(3) Pollak and Curtin, THIS JOURNAL, **72**, 961 (1950).

(4) Pitzer and Beckett, *ibid.*, **69**, 977 (1947).

VAN SLYKE AMINO NITROGEN DETERMINATIONS

Compound	Empirical formula	Van Slyke Nitrogen % Calcd.	Found
d,l-trans-2-Aminocyclohexanol[1b,1c]	$C_6H_{13}NO$	12.15	11.8
d,l-cis-2-Aminocyclohexanol·HCl[1b,1c]	$C_6H_{14}NOCl$	9.23	9.95[a]
cis-(1,3,5)-Inosamine·HCl·H_2O ("SA")[1d]	$C_6H_{16}NO_6Cl$	6.00	6.50[a]
cis-(1,3,5,6)-Inosamine·HCl ("SB")[1d]	$C_6H_{14}NO_5Cl$	6.49	7.60[a]
"Aminodesoxyinositol II"·HCl[e] [*cis*-(1,2,4,5)?]	$C_6H_{14}NO_5Cl$	6.49	7.66[a]
Streptamine·H_2SO_4[d]	$C_6H_{16}N_2O_8S$	10.13	10.03[c]
cis-(1,3,5)-N-Acetylinosamine ("SA")[1d]	$C_8H_{15}NO_4$	6.33[b]	6.13[b]
cis-(1,3,5,6)-N-Acetylinosamine ("SB")[1d]	$C_8H_{15}NO_6$	6.33[b]	6.92[b,a]
d,l-cis-2-Aminocyclopentanol·HCl[1b]	$C_5H_{12}NOCl$	10.23	10.74[a]
Cyclohexanone	$C_6H_{10}O$	0	0.0
Cyclohexene oxide	$C_6H_{10}O$	0	.0
cis-(1,2,3,5)-Inositol	$C_6H_{12}O_6$	0	.0
d,l-cis-2-*p*-Nitrobenzoyloxycyclohexylamine·HCl[1b]	$C_{13}H_{17}N_2O_4Cl$	4.67	5.0

[a] Persistently high results (deviation + 0.5 or greater). [b] After hydrolysis. [c] Reported by Carter, *et al.*, *Science*, **103**, 53 (1946). [d] The diastereomer obtained from streptomycin is presumed to have the *cis*-(1,3,5) configuration, but *cis*-(2,4,5,6) has not been rigorously excluded. See Wolfrom, *et al.*, THIS JOURNAL, **72**, 1724 (1950). [e] Kindly supplied by Dr. H. O. L. Fischer (*vid.*, THIS JOURNAL, **70**, 1479 (1948)).

Godchot and Mousseron.[5] The *cis* epimer gave a 70–80% yield of carbonyl compound ($C_6H_{10}O$), isolated as dinitrophenylhydrazone. The product was found to be a mixture of cyclohexanone and cyclopentylmethanal, apparently richer in the latter. The formation of cyclohexanone was confirmed by isolation of its 2,6-dibenzylidene derivative. (The formation of traces of epoxide or diol has not been excluded.)

The experimental results are thus in qualitative accord with the above predictions.[6]

Anomalous Van Slyke Nitrogen Values.—Some of the aminocyclanols studied gave Van Slyke amino-nitrogen analyses consistently above the theoretical value,[7] while others regularly gave the theoretical result within experimental error.

To determine if the anomalous results were due to reduction of excess nitrous acid by deamination (or pinacol rearrangement) products, control analyses were performed on alicyclic compounds containing ketone, aldehyde, epoxide and *cis* and *trans* diol groups but the result in each case was zero within experimental error.

The aminocyclanols studied are very resistant to deamination by nitrous acid in aqueous mineral acid solutions,[8] but react rapidly with nitrous acid in aqueous acetic acid.

Acknowledgment.—The author is indebted to Dr. D. H. R. Barton of the Imperial College of Science and Technology, London, for a stimulating discussion; and to Dr. H. E. Carter of the University of Illinois for helpful advice and use of laboratory facilities during early stages of the investigation.

Experimental

Melting points (corrected) taken on Köfler micro-block. Microanalyses by Clark Microanalytical Laboratories, Urbana, Ill.

Deamination of *trans*-2-Aminocyclohexanol: Isolation of Cyclopentylmethanal 2,4-Dinitrophenylhydrazone.—A 230-mg. portion of *d,l-trans*-2-aminocyclohexanol[1b,1c] was treated with five equivalents of aqueous sodium nitrite and excess acetic acid at 0–20° for 10 minutes. Aqueous sulfamic acid was then added to decompose excess nitrous acid. Addition of 2,4-dinitrophenylhydrazine solution[9] gave an immediate precipitate. This was filtered and dried, yielding 400 mg. of orange powder, m.p. 150–153°.

Recrystallization from absolute ethanol gave 275 mg. of narrow orange leaflets, m.p. 155–157°. A mixed m.p. with cyclohexanone 2,4-dinitrophenylhydrazone was depressed.

Anal. Calcd. for $C_{12}H_{14}N_4O_4$: C, 51.79; H, 5.07; N, 20.13. Found: C, 51.85; H, 5.02; N, 20.02.

Deamination of *cis*-2-Aminocyclohexanol.—(**A**) Deamination of the *d,l-cis* compound in the above manner yielded 385 mg. of dinitrophenylhydrazone, orange powder, m.p. 126–130°. Recrystallization from ethanol gave yellow-orange crystals, m.p. 132–136°. A second recrystallization gave 255 mg., m.p. 135–140°.

Anal. Calcd. for $C_{12}H_{14}N_4O_4$: C, 51.79; H, 5.07; N, 20.13. Found: C, 51.85; H, 5.31; N, 19.86.

The analysis agrees with either the cyclohexanone or cyclopentylmethanal derivatives. A mixed m.p. with the respective pure derivatives gave in each case an elevation of the m.p., indicating that the product was a mixture of the ketone and aldehyde.

(B).—To 2.0 millimoles of the aminocyclanol hydrochloride was added 4.0 millimoles of anhydrous sodium acetate, 10 millimoles of acetic acid and 1.0 ml. of water. To the clear solution at 25° was added 1.0 ml. of 4 *M* sodium nitrite. The solution became cloudy and developed a pungent odor. After 10 minutes the mixture was steam distilled. To the first 10 ml. of steam distillate was added 2.0 millimoles of 0.13 *M* dinitrophenylhydrazine.[9] The crystals which immediately separated were collected, washed (50% ethanol), and dried, giving 184 mg. of orange crystals, m.p. 120–145°.

After five recrystallizations from absolute ethanol, 40 mg. of nearly pure cyclohexanone 2,4-dinitrophenylhydrazone, m.p. 156–158° was obtained. The mixed m.p. with an authentic sample was raised to 157–159°. A mixed m.p. with the cyclopentylmethanal derivative was depressed to 130–135°.

(5) Godchot and Mousseron, *Compt. rend.*, **198**, 2000 (1934), reported the isolation of cyclopentylmethanal from this reaction as the semicarbazone.

(6) In this connection the results of Bartlett and co-workers (THIS JOURNAL, **60**, 2416 (1938); **59**, 820 (1937)) on the rearrangement of 1,2-dimethylcyclohexanediol-1,2 in dilute sulfuric acid are of interest. The *trans*-diol underwent ring-contraction to give almost exclusively 1-methyl-1-acetylcyclopentane. The *cis*-diol gave almost exclusively 2,2-dimethylcyclohexanone. The analogous *cis*-cyclopentanediol gave the dimethylcyclopentanone, while its *trans* epimer was dehydrated and resinified. These results are at least partially in accord with the above hypothesis.

(7) (a) *Cf.* anomalous high values given by glycine and by cystine, *J. Biol. Chem.*, **117**, 161 (1937). (b) FOOTNOTE ADDED IN PROOF.—The recent studies by A. T. Austin, *J. Chem. Soc.*, 149 (1950), on the mechanism of the Van Slyke deamination of amino-acids may be helpful in explaining the anomalous nitrogen results with amino-alcohols.

(8) Kornblum and Iffland recently reported that aliphatic primary amines do not react with nitrous acid below *p*H 3 (THIS JOURNAL, **71**, 2137 (1949)). Apparently many chemists have used prolonged boiling at low *p*H to force deaminations which would have gone rapidly in the cold at moderate *p*H.

(9) Shriner and Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 97.

The solubility in ethanol at 25° (about 45 mg./100 ml.) of the aldehyde derivative is about twice that of the ketone derivative, suggesting that the aldehyde may actually predominate in the original deamination product.

Isolation of 2-Keto-1,3-dibenzylidenecyclohexane from Deamination Mixtures.—(A) In a control experiment the preparation[10] of 2-keto-1,3-dibenzylidenecyclohexane was carried out in the presence of added sodium nitrite and acetic acid. A 55–70% yield of dibenzalketone of correct m.p. was obtained, indicating that the method should be capable of detecting cyclohexanone in aminocyclanol deamination mixtures, if present.

(B).—A 151-mg. (1 millimole) portion of D,L-*cis*-2-aminocyclohexanol hydrochloride[1b,1c] was treated in the cold with 2.0 millimoles of sodium nitrite, 1.0 millimole of sodium acetate and 9 millimoles of acetic acid, plus water. Sodium hydroxide (10 millimoles), benzaldehyde (2.4 millimoles) and 4 ml. of ethanol were added after 10 minutes. After long standing 37 mg. of yellow crystals, m.p. 116–118°, separated. A mixed m.p. with the dibenzalketone prepared above was not depressed.

Non-reaction of *d,l-cis*-2-Aminocyclohexanol with Nitrous Acid at Low *p*H.—(A) The aminocyclanol hydrochloride was boiled for 1 or 2 minutes with one equivalent of sodium nitrite in excess dilute aqueous hydrochloric acid. Although a peppermint odor indicated a trace of reaction, on benzoylation a high recovery of the starting material as its N-benzoyl derivative, m.p. 187–189°, was obtained.

(B).—The procedure in (A) was modified by using excess sulfuric acid for 0.5 hour at 0°, then boiling the mixture under reflux for 2 hours. Again a high recovery of starting material (N-benzoyl) was obtained.

(10) Vorländer and Kunze, *Ber.*, **59**, 2082 (1926).

TORONTO, CANADA RECEIVED OCTOBER 13, 1950

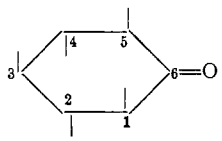
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TORONTO]

N → O Acyl Migration in Epimeric Acetyl Inosamines[1]

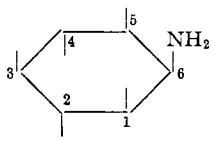
BY G. E. MCCASLAND

(1) The amino-group in *cis*-2-acetaminocyclohexanol is liberated by dilute hydrochloric acid faster than in *trans*. The result is attributed to faster N → O migration in the *cis* epimer. (2) The amino-group of N-acetylinosamine "SA" is liberated faster than that of its epimer "SB," indicating that the "SA" amino-group is *cis* to its two neighboring hydroxyls. (3) The hexacetyl derivative of "aminodesoxyinositol II" has been prepared and is not identical with the hexacetylinosamines "SA," "SB," or "EA." (4) The effect of configuration on the relative basicity of the epimeric 2-aminocyclohexanols is negligible. Each is a weaker base than cyclohexylamine.

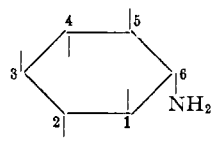
Anderson and Lardy[2] recently reported that the O → N migration is faster in O-acyl derivatives of inosamine-SA than in SB, and that SA is the almost exclusive product of platinum-catalyzed hydrogenation of the oxime of *cis*(1,3,5)-inosose (I). They concluded from these results that the epimer which had been temporarily designated[1d] "SA" has the *cis*(1,3,5,6) configuration (II-A), and that "SB" has the *cis*(1,3,5) configuration (II-B).



I
cis(1,3,5)



II-A
cis(1,3,5,6)
("SA")



II-B
cis(1,3,5)
("SB")

(Hydroxyls to be supplied)

Before learning of Anderson and Lardy's work we had started some experiments on the N → O acyl migration of N-acetylinosamines, and it now seemed desirable to complete these experiments as a check on their proof of configuration.

N → O Migration Mechanisms.—It is well known that the acid-catalyzed deacylation of a 2-acylaminoalkanol (III) in water or alcohol commonly proceeds *via* the 2-acyloxyalkylammonium salt, IV (N → O migration). Isolation of IV is possible when its hydrolysis to the 2-hydroxyalkylammonium salt (V) is slow.[3] Treatment of IV with base liberates the acyloxyalkylamine, which undergoes a rapid reverse migration (O → N) to III. So far as we know the O → N shift always occurs with retention of configuration.

The N → O shift sometimes occurs with inversion, but more commonly with retention. Either VI or VII or their conjugate acids have been proposed[4] as intermediates in the reaction with retention. Welsh[5] has proposed a mechanism for the reaction with inversion.

While our experiments were in progress Fodor and Kiss[6] reported that *cis*-2-benzoylaminocyclohexanol in alcoholic hydrogen chloride gives a more rapid N → O migration than does the corresponding *trans* compound. By O → N migrations with excess base Fodor and Kiss then reconverted each of their amino-ester hydrochlorides back to its respective hydroxy-amide starting material, thus demonstrating retention of configuration for both N → O and O → N migrations under the conditions used (provided one rejects as improbable the occurrence of a single inversion in *both* the forward and reverse migrations.)

If it be assumed that N-acylinosamines in aqueous acid undergo N → O migration and not simple hydrolytic amide cleavage, and that the reaction involves one of the above mechanisms with retention, then an N-acylinosamine with a

(1) For related publications see (a) G. E. McCasland, THIS JOURNAL, **73**, 2293 (1951); (b) McCasland and Smith, *ibid.*, **72**, 2190 (1950); (c) McCasland, Clark and Carter, *ibid.*, **71**, 637 (1949); (d) Carter, Clark, Lytle and McCasland, *J. Biol. Chem.*, **175**, 683 (1948); (e) Carter, Belinskey, Clark, Flynn, Lytle, McCasland and Robbins, *ibid.*, **174**, 415 (1948).

(2) (a) Laurens Anderson, personal communication; (b) L. Anderson and H. A. Lardy, *Abstracts*, Atlantic City Meeting, ACS, Sept., 1949; THIS JOURNAL, **72**, 3141 (1950).

(3) The considerable stability of some 2-acyloxyalkylammonium salts in aqueous acid may be attributed to proton repulsion by the ammonium ion group (away from the carbonyl group).

(4) See Bell, *J. Chem. Soc.*, 2966 (1931); A. P. Phillips and R. Baltzly, THIS JOURNAL, **69**, 200 (1947).

(5) L. Welsh, THIS JOURNAL, **71**, 3500 (1949); **69**, 128 (1947).

(6) G. Fodor and J. Kiss, *Nature*, **164**, 917 (1949); THIS JOURNAL, **72**, 3495 (1950).