[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

Studies on Hygromycin. The Synthesis of Certain Degradation Products of the Antibiotic[1]

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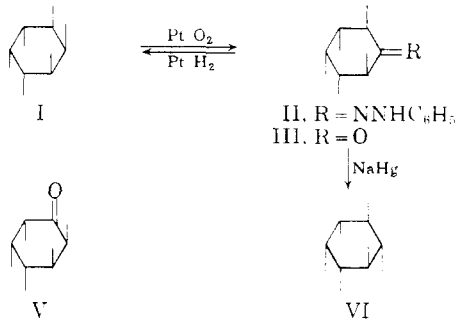
myo-Inosose-5 (III), obtained by catalytic dehydrogenation of neo-inositol (I), was converted into its phenylhydrazone II which on catalytic reduction furnished neo-inosamine-2 (VI). The epimeric *myo*-inosamine-5 (XI) was obtained by sodium amalgam reduction of the sodium oximate of III and by ammonolysis of D-1,2-anhydro-neo-inositol (IX). This last preparation furnished D-allo-inosamine-1 (X) as a co-product. The 3,4-dihydroxy-α-methylcinnamic acid amide (XVI) of neo-inosamine-2 was also prepared. This amide and neo-inosamine-2 were identical with the C_{16}- and C_6-degradation fragments, respectively, of hygromycin.

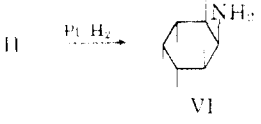
A previous communication[2] reported degradation studies with an antibiotic designated as 1703–18B in these laboratories. This antibiotic is identical with the antibiotics hygromycin[3] and homomycin.[3,4] Acid hydrolysis of hygromycin afforded a $C_6H_{13}NO_5$ basic fragment,[5] which was demonstrated to be neo-inosamine-2 (VI)[8] through a series of elegant experiments by Patrick and his co-workers.[2] In the present paper we wish to describe an unequivocal synthesis of VI and its 3,4-dihydroxy-α-methylcinnamic acid amide, another degradation product of hygromycin.[3,6,7]

The most direct method for preparing VI appeared to be the reduction of an appropriate inosose hydrazone or oxime. However, such an approach required the heretofore unknown *myo*-inosose-5 (III).[9] Although preliminary efforts to effect the preparation of this inosose by nitric acid oxidation of neo-inositol (I)[10] were unrewarding, catalytic dehydrogenation[11] of this inositol gave an inosose, isolated as its phenylhydrazone, in 23–47% yield. Treatment of this derivative with benzaldehyde in the presence of benzoic acid[12] regenerated the inosose in 60–76% yield. Hydrogenation of this inosose in the presence of platinum furnished neo-inositol[10] in 88% yield, whereas reduction with sodium amalgam gave *myo*-inositol (IV), isolated as its hexaacetate, in 40% yield. Of the two structures (III and V) possible for an inosose derived from I, only that of *myo*-inosose-5 (III) is compatible with the results of these reduction experiments. Thus, dehydrogenation occurred preferentially at an axial hydroxyl group, an observation which was noted in previous studies.[11]



Of interest is that catalytic dehydrogenation of neo-inositol, which contains two axial hydroxyl groups, ceases at the monoketone stage, for subsequent studies showed that dehydrogenation of this inositol with *Acetobacter suboxydans* proceeds to 2,5-diketo-*myo*-inositol.[13] Following the completion of this work it was reported that platinum-catalyzed dehydrogenation of inositols is a highly stereospecific reaction in which only axial hydroxyl groups are affected.[14] Moreover, our observation that the catalytic dehydrogenation of an inositol containing two axial hydroxyl groups ceases at the monoketone stage has since been confirmed by other workers with L-inositol, D-inositol, (+)-pinitol and quebrachitol.[14c]

With the proper inosose available, neo-inosamine-2 (VI) was readily obtained. Thus, the phenylhydrazone II was hydrogenated in glacial acetic acid in the presence of a platinum catalyst to give an



inosamine in 53% yield. Since previous workers have reported that reduction under such conditions results in predominant introduction of an axial amino group,[15] it was postulated that this product was neo-inosamine-2 (VI).[16]

(1) A portion of this work was the subject of a preliminary communication [*J. Am. Chem. Soc.*, **78**, 5691 (1956)].

(2) J. B. Patrick, R. P. Williams, C. W. Waller and B. L. Hutchings, *ibid.*, **78**, 2652 (1956).

(3) J. B. Patrick, private communication.

(4) K. Isono, S. Yamashita, Y. Tomiyama, S. Suzuki and H. Sakai, *J. Antibiotics Japan*, (**A**) **10**, 21 (1957).

(5) More extensive degradative experiments are described by Mann and Woolf[6] and the Japanese group.[7]

(6) R. L. Mann and D. O. Woolf, *J. Am. Chem. Soc.*, **79**, 120 (1957).

(7) M. Namiki, K. Isono and S. Suzuki, *J. Antibiotics Japan*, (**A**) **10**, 160 (1957).

(8) The system of nomenclature used in this paper is that proposed by Fletcher and his associates [H. G. Fletcher, Jr., L. Anderson and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951)].

(9) In our earlier communication this compound was erroneously designated "neo-inosose-2."

(10) S. J. Angyal and N. K. Matheson, *J. Am. Chem. Soc.*, **77**, 4343 (1955).

(11) Method of K. Heyns and H. Paulsen, *Ber.*, **86**, 833 (1953); **89**, 1152 (1956).

(12) T. Posternak, *Helv. Chim. Acta*, **19**, 1333 (1936).

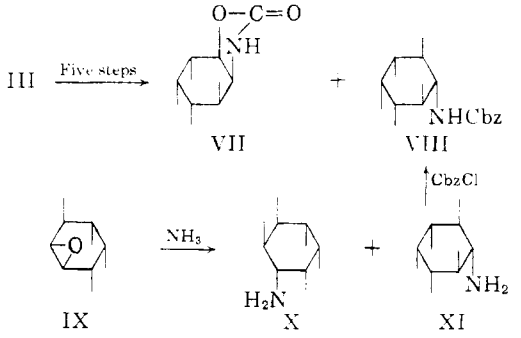
(13) L. Anderson, R. Takeda, S. J. Angyal and D. J. McHugh, *Arch. Biochem. Biophys.*, **78**, 518 (1958).

(14) (a) S. J. Angyal, *Quart. Revs.*, **11**, 212 (1957); (b) L. Anderson, E. S. DeLuca, A. Bieder and G. G. Post, *J. Am. Chem. Soc.*, **79**, 1171 (1957); (c) G. G. Post and L. Anderson, *ibid.*, **84**, 471 (1962). The author is indebted to Professor Angyal for communicating this observation prior to its publication.

(15) (a) L. Anderson and H. H. Lardy, *J. Am. Chem. Soc.*, **72**, 3141 (1950); (b) E. L. May and E. Mosettig, *J. Org. Chem.*, **14**, 1137 (1949); (c) T. Posternak, *Helv. Chim. Acta*, **33**, 1597 (1950).

(16) In a private communication Professor S. J. Angyal has informed the author that he, too, has carried out the catalytic dehydro

Unequivocal proof for the configuration of VI was obtained by the preparation of *myo*-inosamine-5 (XI), which is epimeric with VI at the carbon bearing the amino group. The synthesis of XI was accomplished in two ways. Initially, *myo*-inosose-5 (III) was converted into its sodium oximate which, without isolation, was reduced with sodium amalgam.[17,17a] The reaction mixture was acetylated, and the epimeric hexaacetates thus obtained were completely deacetylated with hydrochloric acid. Treatment of the epimeric inosamines with carbobenzyloxy chloride, under Schotten–Bauman conditions, furnished in low yields the cyclic carbamate VII[2] of neo-inosamine-2 and the N-carbobenzyloxy derivative of a new inosamine. This latter product was considered to have the



5-carbobenzyloxyamino-5-deoxy-*myo*-inositol structure VIII.[19] The second preparation of *myo*-inosamine-5 (XI) proceeded from D-1,2-anhydro-neo-inositol (IX).[20]

Ammonolysis[21] of this oxide furnished a mixture of inosamines which was resolved by chromatography on powdered cellulose into crystalline inosamine A (10% yield) and amorphous inosamine B (70% yield). The latter was characterized as its crystalline hexaacetyl (obtained in 83% yield) and N-acetyl derivatives. By virtue of its optical inactivity, inosamine A must be *myo*-inosamine-5 (XI). This conclusion was verified by conversion of inosamine A into its N-carbobenzyloxy derivative. The latter was identical by the usual criteria with the N-carbobenzyloxyinosamine prepared from *myo*-inosose-5 (see above). Since the only possible common structure for an inosamine derived from the inosose III and the oxide IX is *myo*-inosamine-5 (XI), the assignment of this configuration to inosamine A receives unequivocal support. By elimination, inosamine B must then be D-allo-inosamine-1 (X), a conclusion supported by the optical activity of its derivatives. Moreover, the inosamine prepared by catalytic reduction of *myo*-inosose-5 phenylhydrazone (II) must indeed be neo-inosamine-2 (VI). This inosamine and the $C_6H_{13}NO_5$ fragment which had been isolated from hygromycin were identical according to the usual criteria, as were their hexaacetate and N-benzylidene derivatives.

It may be noted that the relative proportion of *myo*-inosamine-5 (XI) and D-allo-inosamine-1 (X) obtained on ammonolysis of oxide IX is that which would be predicted by conformational considerations.[14a] Moreover, the synthesis of *myo*-inosamine-5 (XI) and neo-inosamine-2 (VI) completes the synthesis of all possible position-isomers of the inosamines having the *myo*-[22] and neo-configurations.[23]

Mercaptanolysis[6,7] or ion-exchange resin-catalyzed hydrolysis[3] of hygromycin had given a C_{16}-$H_{21}NO_8$ product. In view of its spectral properties and its hydrolysis to neo-inosamine-2 (VI) and 3,4-dihydroxy-α-methylcinnamic acid, it was postulated that this product was the amide XVI.[3,6,7] Confirmation of structure XVI as that of the C_{16}-degradation fragment was furnished by an unequivocal synthesis of XVI. Several attempts to effect N-acylation of model inosamines by certain cinnamoyl halides by the Schotten–Bauman and other procedures were unsuccessful. From these experiments only starting material could be isolated. The synthesis of XVI finally was achieved *via* the acylation of penta-*O*-acetyl-neo-inosamine-2 hydrochloride (XIV), which was obtained in the following manner. Acetylation[25] of N-benzylidene-neo-inosamine-2 (XII) gave its penta-*O*-acetyl derivative XIII which was preferentially hydrolyzed to penta-*O*-acetyl-neo-inosamine-2 hydrochloride (XIV) using hydrochloric acid in tetrahydrofuran. In the latter reaction the choice of solvent proved to be a critical factor. Thus, use of dioxane as a reaction medium gave only the hydrochloride of neo-inosamine-2, presumably because of the solubility of XIV in this medium. However, with tetrahydrofuran as the reaction medium, XIV could be isolated in 73% yield, for it separated

genation of neo-inositol (I) to give *myo*-inosose-5 (III), the phenylhydrazone of which was catalytically reduced to furnish neo-inosamine-2 (VI).

(17) The tendency of this reagent to produce mixtures consisting mainly of the epimer wherein the newly-formed substituent is equatorial is well known.[12,15,18]

(17a) NOTE ADDED IN PROOF.—A recent paper by M. Nakajima and his associates [*Ber.*, **95**, 141 (1962)] also described the preparation of *myo*-inosamine-5. Our syntheses of this inosamine are identical with theirs.

(18) H. Straube-Rieke, H. A. Lardy and L. Anderson, *J. Am. Chem. Soc.*, **75**, 694 (1953).

(19) Although the distance between 1,2-*cis*(*e*,*p*)-substituents is the same as that between 1,2-*trans*(*e*,*e* only)-substituents, no example is known wherein an inosamine with a 1,2(*e*,*e*)-aminohydroxy system reacts with carbobenzyloxy chloride to yield a cyclic carbamate. This difference in reactivity may be explained readily by the resistance in a *trans*(*e*,*e*)-system to the compression of axial groups that would be prerequisite to the fusion of a five-membered ring with the cyclohexane ring. This explanation was first advanced by Angyal[14a] to account for the observation that *e*,*e*-glycol systems do not form isopropylidene derivatives with acetone.

(20) This oxide was obtained from D-1,2-anhydro-3,4;5,6-di-*O*-isopropylidene-allo-inositol using the procedure described by Angyal and Gilham [*J. Chem. Soc.*, 3691 (1957)] for this conversion in the enantiomorphic series.

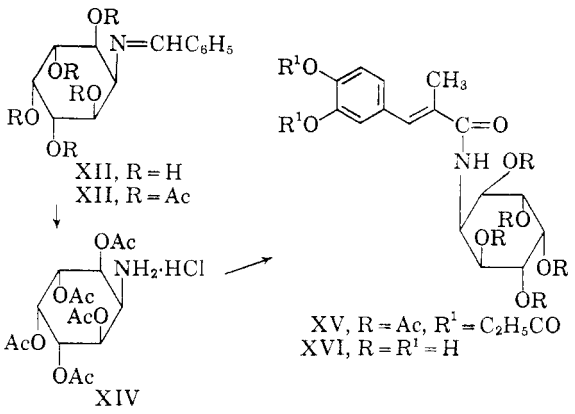
(21) (a) L. Anderson, Abstracts of the 130th Meeting of the American Chemical Society, Atlantic City, N. J., September, 1956, p. 27-D; (b) G. R. Allen, Jr., *J. Am. Chem. Soc.*, **79**, 1167 (1957).

(22) The synthesis of DL-*myo*-inosamine-4,[18] L-*myo*-inosamine-1[24b] and *myo*-inosamine-2[24a] has been described by other workers.

(23) L-Neo-inosamine-1 has been prepared previously.[21]

(24) (a) H. E. Carter, R. K. Clark, Jr., B. Lytle and G. E. McCasland, *J. Biol. Chem.*, **175**, 683 (1948); (b) see refs. 15a and 15c.

(25) The choice of *O*-acetyl blocking groups was made as the result of a conversation with Dr. J. B. Patrick. He and his co-workers attempted a partial synthesis of amide XVI using essentially the same method described herein; their procedure differed only in that they chose *O*-benzoyl blocking groups. Although the sequence proceeded smoothly to the *O*-benzoyl analog of XV (R = Bz, R^1 = C_2H_5CO), it was found that de-*O*-acylation of this blocked amide was accompanied by acyl migration and expulsion of the original N-acyl group, giving 2-benzamido-2-deoxy-neo-inositol as the sole product.



from solution as it was formed. The blocked amine XIV reacted with α-methyl-3,4-dipropionoxycinnamoyl chloride to give the blocked amide XV in 69% yield. De-*O*-acylation of the latter with triethylamine in methanol furnished the desired amide XVI in 71% yield. This amide proved to be identical with the $C_{16}H_{21}NO_8$ degradation product of hygromycin.

Acknowledgment.—The author is indebted to Drs. J. B. Patrick and H. M. Kissman for many helpful discussions. Without a generous gift of pinitol from Dr. Arthur B. Anderson of the Forest Products Laboratory, University of California, Richmond, Calif., this investigation would not have been possible. The assistance of Mr. J. Poletto, who performed the large-scale preparation of starting materials, and Dr. M. J. Weiss, who aided in the preparation of this manuscript, is gratefully acknowledged. Microanalyses were carried out by Mr. L. Brancone and staff and spectroscopic and polarimetric work by Mr. W. Fulmor and staff.

Experimental[26]

***myo*-Inosose-5 Phenylhydrazone (II).**—A mechanically stirred solution of 2.855 g. (15.8 mmoles) of neo-inositol[10,27] in 700 ml. of water was heated to 70°, and treated with a mixture obtained by catalytic reduction of 1.86 g. of platinum oxide and 35 ml. of water. A vigorous stream of oxygen was introduced for 30 min. while the temperature was maintained at 65–70°.[28] The mixture was filtered through a bed of Celite,[29] the filtrate was concentrated to a volume of 75 ml. and treated with a solution of 3.6 ml. of phenylhydrazine in 5.6 ml. of 50% acetic acid. The solution became red; cooling and vigorous scratching induced crystallization. Filtration gave a solid which was washed with ethanol and then ether to give 1.500 g. (33% yield) of pink solid, m.p. 195–197° dec.

The phenylhydrazone was recrystallized from methanol to give white crystals, m.p. 201–204° dec.; λ_{max}^{MeOH} 278 mμ (ε 18,200); λ_{max}^{KBr} 3.05, 6.07, 6.25, 6.66 μ.

Anal. Calcd. for $C_{12}H_{16}N_2O_5$: C, 53.72; H, 6.01; N, 10.44. Found: C, 53.68; H, 6.21; N, 10.53.

A preliminary experiment gave the phenylhydrazone in 47% yield.

***myo*-Inosose-5 (III).**—A mixture of 0.730 g. (2.62 mmoles) of *myo*-inosose-5 phenylhydrazone (II), 0.050 g. of benzoic acid, 1 ml. of benzaldehyde and 15 ml. of water was heated at reflux for 1 hr. The cool mixture was extracted with ether, and the ether extracts were discarded. The aqueous solution was treated with a drop of concentrated sulfuric acid solution and evaporated to near dryness. Trituration of the residue with 5 ml. of water gave a solid which was recrystallized from water to give 0.320 g. (69% yield) of white crystals, m.p. 218–220° dec., $\lambda_{max}^{H_2O}$ 284 mμ (ε 86), λ_{max}^{KBr} 3.00 μ.

Anal. Calcd. for $C_6H_{10}O_6$: C, 40.45; H, 5.66. Found: C, 40.49; H, 5.42.

In a subsequent experiment, *myo*-inosose-5 was obtained in 76% yield.

Reduction of *myo*-Inosose-5 (III). A. With Platinum and Hydrogen.—A mixture of 0.050 g. of platinum oxide and 25 ml. of water was reduced with hydrogen, treated with 0.100 g. (0.56 mmole) of the inosose and magnetically stirred under hydrogen. A decrease of 12 ml. (S.T.P.) (95%) in the volume of hydrogen was noted. The mixture was filtered, and the water-white filtrate was evaporated to a volume of *ca.* 5 ml. The concentrate was chilled and filtered to give 0.089 g. (88% yield) of needles, m.p. 314° when dropped on a preheated hot-stage. The infrared spectrum of this material was identical with that of a known sample of ***neo*-inositol (I).**

Anal. Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.71. Found: C, 40.05; H, 6.91.

When treated with sodium acetate and acetic anhydride, this material gave **neo-inositol hexaacetate** in 62% yield as white crystals, m.p. 252–253° (reported[10] 253°). The identity of this material was established by mixture melting point and infrared spectral comparisons.

Anal. Calcd. for $C_{18}H_{24}O_{12}$: C, 50.00; H, 5.60. Found: C, 50.28; H, 5.83.

B. With Sodium Amalgam.—A solution of 0.100 g. (0.56 mmole) of the inosose in 25 ml. of boiling water was cooled to 40°. The *p*H of the solution was adjusted to 5.5 by the addition of glacial acetic acid. Sodium amalgam (2 g.) was added with stirring, the *p*H of the mixture was maintained at 5.5–6.5 by the addition of glacial acetic acid, and the temperature was kept at 35–45°. After the evolution of gas had ceased, additional sodium amalgam (4 g.) was added in two equal portions. The aqueous phase was decanted, and the mercury was washed with water. The combined aqueous solutions were taken to dryness, and the residue was acetylated by the sodium acetate–acetic anhydride method. The product was triturated with 15 ml. of boiling ethanol and recrystallized from ethanol to give 0.094 g. (40% yield) of ***myo*-inositol hexaacetate** as white crystals, m.p. 212–213°. Mixture melting point and infrared spectral comparisons established the identity of this material.

Anal. Calcd. for $C_{18}H_{24}O_{12}$: C, 50.00; H, 5.60; Found: C, 50.34; H, 5.69.

Neo-inosamine-2 (VI).—A mixture of 0.536 g. (2.00 mmoles) of *myo*-inosose-5 phenylhydrazone (II) and 0.300 g. of platinum oxide in 100 ml. of glacial acetic acid was shaken under hydrogen. The drop in pressure ceased after 10 min. The mixture was filtered through Celite,[29] and the residue washed with acetic acid. The combined acetic acid solutions were decolorized and evaporated to give an amber sirup which on trituration with ethanol gave white crystals. The mixture was filtered, and the tacky solid was instantly dissolved in 25 ml. of water and stirred with 10 g. of Amberlite IRA-400 (OH)[30] for 30 min. The mixture was filtered, and the filtrate was taken to a volume of 5 ml. On cooling the concentrate, white crystals separated. This material was recrystallized from water–acetone to give 0.190 g. (56% yield) of white crystals, m.p. 239–241° dec. A mixture of this material with the inosamine isolated from hydromycin melted at 238–240° dec. Moreover, the infrared spectra of the synthetic and natural inosamines were identical.

Anal. Calcd. for $C_6H_{13}NO_5$: C, 40.22; H, 7.31; N, 7.82. Found: C, 40.41; H, 7.73; N, 7.84.

(26) All melting points were determined on a Kofler hot-stage and are corrected; evaporations were carried out under reduced pressure.

(27) The neo-inositol was prepared from D-inositol, obtained by the demethylation of pinitol, using the procedure applied by Angyal and co-workers [*J. Chem. Soc.*, 686 (1952); ref. 10] to the conversion of the enantiomorphic L-inositol into neo-inositol.

(28) It is conceivable that a shorter reaction time would have given a somewhat greater yield, since reactions conducted under the above conditions for 45 and 120 minutes gave the phenylhydrazone in 28 and 23% yield, respectively. The limited amount of neo-inositol available prohibited clarification of this point.

(29) Celite is Johns-Manville's registered trade-mark for diatomaceous silica products.

(30) A synthetic anion exchange resin of the modified amine type produced by Rohm and Haas Co.

Acetylation of synthetic neo-inosamine-2 by the sodium acetate–acetic anhydride method gave a hexaacetate as white crystals, m.p. 277–278° after recrystallization from ethanol. A mixture with neo-inosamine-2 hexaacetate from the natural inosamine melted at 277–278°. Additionally, the infrared spectra of the two samples were identical.

Anal. Calcd. for $C_{18}H_{23}NO_{11}$: C, 50.11; H, 5.84; N, 3.25. Found: C, 50.35; H, 6.02; N, 3.30.

N-Benzylidene-neo-inosamine-2 (XII).—A mixture of 0.600 g. (0.335 mmole) of synthetic neo-inosamine-2, 0.5 ml. of benzaldehyde and 25 ml. of ethanol was heated at reflux temperature for 8 hr. All solid dissolved within this time. The hot solution was filtered, and the filtrate was evaporated to a volume of about 10 ml. Chilling of the concentrate gave 0.070 g. (78% yield) of crystals, m.p. 209–211° dec. after a crystalline change to needles at 94°; comparison with a sample from natural neo-inosamine-2 by mixture melting point and infrared spectra showed the identity of the two samples.

Anal. Calcd. for $C_{13}H_{17}NO_6$: C, 58.41; H, 6.41; N, 5.24. Found: C, 58.77; H, 6.49; N, 5.12.

In two other experiments, utilizing the inosamine isolated from hygromycin, an average yield of 92% was obtained.

5-Carbobenzyloxyamino-5-deoxy-*myo*-inositol (VIII). A. From *myo*-Inosose-5 (III).—A solution of 0.424 g. (6.1 mmoles) of hydroxyamine hydrochloride in 10.8 ml. of 1 *N* sodium hydroxide solution was treated with 0.737 g. (4.15 mmoles) of *myo*-inosose-5 (III); the mixture was allowed to stand at room temperature for 2 hr. during which time all of the solid dissolved. The *p*H of the resulting solution was adjusted to 5.5 by the addition of glacial acetic acid. The temperature of the reaction solution was kept at 25–30°, and there was added in five equal portions 20 g. of sodium amalgam. The *p*H of the reaction mixture was maintained at 5.5–6.5 by the addition of glacial acetic acid. The aqueous solution was decanted from the mercury which was washed with water. The combined aqueous solutions were filtered through Celite,[29] and the filtrate was taken to near dryness. The moist residue was dried under reduced pressure over phosphoric anhydride.

The residue was acetylated by the sodium acetate–acetic anhydride method to give 0.425 g. of epimeric inosamine hexaacetates.

This mixture was heated on the steam-bath with 10 ml. of 6 *N* hydrochloric acid solution for 2 hr. The resulting solution was decolorized and taken to dryness. Ethanol was added and removed in the usual manner to give a glass which was dissolved in 5 ml. of 1 *N* sodium hydroxide solution. This solution was chilled and treated with 1.0 ml. of a solution of carbobenzyloxy chloride in toluene (5.2 mmoles of acyl halide/ml.). The mixture was magnetically stirred for 90 min. and then allowed to stand at room temperature for 5 days. At the end of this period most of the toluene had evaporated and solid had separated. The mixture was triturated with ether and filtered to give 0.112 g. (9% yield) of VIII as buff-colored crystals, m.p. 228–230° dec. This material was recrystallized from water to give white crystals, m.p. 238–240° dec.; λ_{max}^{KBr} 3.00, 5.95, 6.43, 7.97, 9.52 and 14.37 μ.

Anal. Calcd. for $C_{14}H_{19}NO_7 \cdot {}^1/_2H_2O$: C, 52.16; H, 6.26; N, 4.35. Found: C, 52.00; H, 6.50; N, 4.29.

The two-phase filtrate from the separation of this material was separated, and the aqueous solution was stirred with 10 g. of Amberlite IR-120 (H)[31] and 50 ml. of water. The mixture was filtered, and the filtrate was taken to dryness. Crystallization of the residue from dilute alcohol gave 60 mg. (7% yield) of DL-*N*,*O*^{1}-carbonyl-neo-inosamine-2(VII) as white crystals, m.p. 207–209° dec. (reported[2] 203–205°).

B. From *myo*-Inosamine-5 (XI).—A mixture of 21 mg. (0.12 mmole) of *myo*-inosamine-5 (XI) (prepared from D-1,2-anhydro-neo-inositol as described below), 1 ml. of 1 *N* sodium hydroxide solution and 0.5 ml. of a solution of carbobenzyloxy chloride in toluene (0.52 mmole of reagent/ml.) was stirred at room temperature for 1 hr. and allowed to stand at room temperature for 4 days. The solid which had separated was collected by filtration to give 17 mg. (45%) yield) of white crystals, m.p. 238–240° dec. alone or when in mixture with the material obtained in method A. Furthermore, the infrared spectra of the two samples were identical.

(31) A synthetic cation exchange resin of the nuclear sulfonic acid type produced by Rohm and Haas Co.

Reaction of D-1,2-Anhydro-neo-inositol (IX) with Ammonia.—A mixture of 0.500 g. (3.0 mmoles) of D-1,2-anhydro-neo-inositol (IX)[20] and 50 ml. of methanol saturated with ammonia at 10° was heated in a stainless steel bomb on the steam-bath for 20 hr. The contents of the bomb was filtered through a bed of Celite[29]; the bomb was rinsed with two 50-ml. portions of methanol and these rinsings were filtered in the same manner. The combined filtrate and washings were taken to dryness, and the residue was dissolved in 7 ml. of water. This solution was diluted with 7 ml. of acetone and placed on a column prepared from powdered cellulose[32] (column size 2.8 × 35 cm.). The column was eluted with acetone–water (4:1), and 197 10-ml. fractions were collected. The material contained in fractions 51–80 was combined, and the solvent was removed to give 0.379 g. (70% yield) of a white glass; paper chromatography in acetone–water (4:1) showed a single spot with R_f 0.44. This material had $[\alpha]^{25}D$ +22.7° (*c* 1.59, water) and consequently was considered to be D-allo-insoamine-1 (X).

Fractions 95–115 were taken to dryness, and the solid residue showed two spots with R_f 0.32 and 0.45 upon paper chromatography in acetone–water (4:1). Nevertheless two recrystallizations of this residue from water–acetone gave 57 mg. (10% yield) of *myo*-inosamine-5 (XI) as white crystals which slowly decomposed and sublimed from 235° but failed to melt below 300°. Paper chromatography of this material in acetone–water (4:1) showed a single spot with R_f 0.32, and the material had $[\alpha]^{25}D$ 0° (*c* 0.21, 5% ammonium molybdate in water).[33]

Anal. Calcd. for $C_6H_{13}NO_5$: C, 40.22; H, 7.31; N, 7.82. Found: C, 40.03; H, 7.20; N, 7.95.

D-Allo-inosamine-1 Hexaacetate.—Acetylation of 0.200 g. (1.1 mmoles) of D-allo-inosamine-1 by the sodium acetate–acetic anhydride method gave after two recrystallizations from water 0.400 g. (83% yield) of white crystals, m.p. 233–235°, $[\alpha]^{25}D \sim +2°$ (*c* 0.53, chloroform); λ_{max}^{KBr} 5.70, 6.08, 6.39, 8.15 (broad) and 9.30 μ.

Anal. Calcd. for $C_{18}H_{25}NO_{11}$: C, 50.11; H, 5.84; N, 3.25. Found: C, 50.26; H, 5.89; N, 3.27.

D-1-Acetamido-1-deoxy-allo-inositol.—A solution of 0.100 g. (0.23 mmole) of D-allo-inosamine-1 hexaacetate and 5 ml. of methanol saturated with ammonia at 10° was allowed to stand at room temperature for 7 days at which time all solvent had evaporated. The amorphous residue was dissolved in 3 ml. of ethanol and crystallization induced by scratching to afford 38 mg. (75% yield) of white crystals, m.p. 209–211° dec., $[\alpha]^{25}D$ + 40.8° (*c* 1.2, water); λ_{max}^{KBr} 2.88, 2.95, 3.05, 6.08, 6.44, 8.00, 9.25 μ (broad).

Anal. Calcd. for $C_8H_{15}NO_6$: C, 43.44; H, 6.84. Found: C, 43.21; H, 6.40.

Penta-*O*-acetyl-*N*-benzylidene-neo-inosamine-2 (XIII).—Acetylation of 0.500 g. (1.87 mmoles) of N-benzylidene-neo-inosamine-2 (XII) by the pyridine–acetic anhydride method gave 0.518 g. (58% yield) of white crystals, m.p. 213–215°. Two recrystallizations from ethanol gave flat white needles, m.p. 217–219°, λ_{max}^{KBr} 5.71 and 6.05 μ.

Anal. Calcd. for $C_{23}H_{27}NO_{10}$: C, 57.86; H, 5.70; N, 2.93. Found: C, 57.61; H, 6.09; N, 3.10.

In a second experiment a 79% yield of the material was obtained.

Penta-*O*-acetyl-neo-inosamine-2 Hydrochloride (XIV).—A solution of 0.250 g. (0.525 mmole) of penta-*O*-acetyl-N-benzylidene-neo-inosamine-2 (XIII) in 10 ml. of tetrahydrofuran was treated with 0.5 ml. of 6 *N* hydrochloric acid solution. After the solution remained at room temperature during 3 min., crystals began separating. After 1 hr. an additional 10 ml. of tetrahydrofuran was added, and an hour later the solid was collected by filtration to give 0.123 g. (55% yield) of white crystals, m.p. 185–187°, λ_{max}^{KBr} 2.92 and 5.68 μ.

Anal. Calcd. for $C_{18}H_{23}NO_{10} \cdot HCl$: C, 45.13; H, 5.68; N, 3.29. Found: C, 45.39; H, 6.04; N, 3.40.

In a second experiment a 73% yield of this material was obtained.

1,3,4,5,6-Penta-*O*-acetyl-2-deoxy-2-(α-methyl-3,4-dipropionoxycinnamido)-neo-inositol (XV).—A solution of 0.306

(32) The powdered cellulose was Whatman standard grade and was used as received.

(33) W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948, p. 248.

g. (1.0 mmole) of α-methyl-3,4-dipropionoxycinnamic acid in 15 ml. of thionyl chloride was allowed to reflux on the steam-bath for 2 hr. The excess thionyl chloride was removed by evaporation, and 5 ml. of benzene was added. The benzene was removed by evaporation. The benzene addition and removal was twice repeated. The resulting acid chloride was dissolved in 10 ml. of chloroform.

A solution of 0.426 g. (1.0 mmole) of penta-*O*-acetyl-neo-inosamine-2 hydrochloride (XIV) and 0.202 g. (2.0 mmoles, 0.28 ml.) of triethylamine in 20 ml. of chloroform was magnetically stirred with the acid chloride solution for 2 hr. The chloroform solution was washed with water, dried over magnesium sulfate, and evaporated to give a gum which was dissolved in 15 ml. of ethanol. The ethanol solution was concentrated at atmospheric pressure to 5-ml. volume and diluted with 5 ml. of water. This precipitated a gum which crystallized on scratching to give 0.517 g. (76% yield) of cream-colored crystals, m.p. 157–160° after two recrystallizations from dilute alcohol. The material had λ_{max}^{MeOH} 209 (ε 20,600) and 260 mμ (ε 18,700); λ_{max}^{HCl} 209 (ε 21,100) and 260 mμ (ε 19,300); λ_{max}^{NaOH} 252 (ε 12,900) and 316 mμ (ε 7,130); λ_{max}^{KBr} 2.96, 5.69, 5.98, 6.10, 6.56(shoulder), 6.63, and 8.13 μ.

Anal. Calcd. for $C_{32}H_{39}NO_{15}$: C, 56.71; H, 5.80; N, 2.07. Found: C, 56.83; H, 5.60; N, 2.16.

In a second experiment this product was obtained in 69% yield.

2-Deoxy-2-(3,4-dihydroxy-α-methylcinnamido)-neo-inositol (XVI).—A solution of 0.465 g. (0.69 mmole) of 1,3,4,5,6-penta-*O*-acetyl-2-deoxy-2-(α-methyl-3,4-propionoxycinnamido)-neo-inositol (XV) and 0.146 g. (1.45 mmoles, 0.20 ml.) of triethylamine in 25 ml. of anhydrous methanol was allowed to reflux for 2 hr. The solution was acidified with glacial acetic acid and evaporated. The amber residual sirup was treated with 1.5 ml. of glacial acetic acid and allowed to stand at room temperature for 10 min. during which time crystals separated. The mixture was diluted with 5 ml. of acetone and filtered to give 0.175 g. (71% yield) of white crystals, m.p. 248–252° dec. This material was recrystallized from water to give buff-colored crystals, m.p. 256–259° dec. A mixture of this material with the $C_{16}H_{21}NO_8$ degradation product (m.p. 253–258° dec.) of the antibiotic hygromycin melted at 253–259° dec. Furthermore, the infrared spectra of the two samples were identical, and the ultraviolet spectra of the degradation product and the synthetic material were essentially the same.

Anal. Calcd. for $C_{16}H_{21}NO_8$: C, 54.08; H, 5.96; N, 3.94. Found: C, 53.71; H, 6.01; N, 4.06.

Paper Chromatography.—Circular paper chromatograms were run in the apparatus described by Kawerau.[34] The apparatus (26-cm. diameter) was purchased from the Shandon Scientific Co., London, Eng., and modified in the manner described by Kissman and Weiss.[35] The paper used was a special Whatman #1 filter paper (KCT-26) which had been slotted for the Kawerau apparatus. Solvents were mixed just before use, and the paper was not equilibrated with the solvent mixture. The inosamine derivatives were detected with the silver nitrate–sodium hydroxide reagent described by Trevelyan, *et al.*,[36] as modified by Anet and Reynolds,[37] and the chromatograms were fixed by spraying with thiosulfate.[38]

(34) E. Kawerau, *Chromatographic Methods*, **1**, No. 2, 7 (1956) [published by H. Reeve Angel and Co., 52 Duane Street, New York 7, N. Y.].

(35) H. M. Kissman and M. J. Weiss, *J. Am. Chem. Soc.*, **80**, 5559 (1958).

(36) W. E. Trevelyan, D. P. Proctor and J. S. Harrison, *Nature*, **166**, 444 (1950).

(37) E. F. L. J. Anet and T. M. Reynolds, *ibid.*, **174**, 930 (1954).

(38) S. J. Angyal, D. J. McHugh and P. T. Gilham, *J. Chem. Soc.*, 1432 (1957).

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Stereochemistry and the Mechanism of Hydrogenation of Cyclo-alkenes.[1] IV. 4-*tert*-Butyl-1-methylcyclohexene and 4-*tert*-Butyl-1-methylenecyclohexane on Platinum Oxide and a Palladium Catalyst[2]

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The ratio of the saturated stereoisomers obtained upon the hydrogenation of 4-*tert*-butyl-1-methylcyclohexene (I) and 4-*tert*-butyl-1-methylenecyclohexane (II) on reduced PtO_2 is a function of the pressure of hydrogen. The *tert*-butyl group magnifies the steric effects over those previously observed. A simple mathematical analysis of the previously proposed mechanistic scheme is shown to be consistent with the stereochemical information. The characteristics of the reaction in the presence of a palladium catalyst are also accounted for with the assumption that the rate-limiting surface reaction occurs at a later stage than that which pertains on platinum.

Previous stereochemical studies of the hydrogenation of 1,2-dimethylcyclohexene and several of its isomers on platinum[4] and palladium[1] catalysts had suggested that the distribution of the saturated stereoisomers, as well as the isomeric cycloalkenes formed concurrently, may serve to identify the product and/or rate-controlling surface reaction and also to define the geometry of the pertinent transition states. Arguments based upon conformational theory were employed and the treatment of mechanism was qualitative. In the present work, 4-*tert*-butyl-1-methylcyclohexene and 4-*tert*-butyl-1-methylenecyclohexane have been hydrogenated in the liquid phase (acetic acid solvent) and in contact with reduced PtO_2. The bulk of the *tert*-butyl group restricts the conformations of the six-membered cycle to which it is attached to those in which this group is equatorial or quasi equatorial[5]; consequently, conformational effects and analysis are simplified, and the resulting stereochemistry is more readily identified with a simple, mathematical treatment of the proposed mechanistic scheme.

Experimental

Preparation of 4-*tert*-Butyl-1-methylenecyclohexane.—4-*tert*-Butyl-1-methylenecyclohexane was prepared by the pyrolysis of the unsaturated acid obtained *via* the Reformatsky reaction of 4-*tert*-butylcyclohexanone and ethyl bromoacetate.[6,7] The crude olefin, b.p. 185–187° (730 mm.)

(1) For the previous paper in this series, see S. Siegel and G. V. Smith, *J. Am. Chem. Soc.*, **82**, 6087 (1960).

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(3) Taken in part from the M.S. thesis of B. D., January, 1960.

(4) S. Siegel and G. V. Smith, *J. Am. Chem. Soc.*, **82**, 6082 (1960).

(5) S. Winstein and N. S. Holness, *ibid.*, **77**, 5562 (1955).

(6) J. W. Baker and J. B. Holdsworth, *J. Chem. Soc.*, 728 (1945).

(7) B. Cross and G. H. Whitham, *ibid.*, 3892 (1960).