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SYNTHESIS OF AMINOCYCLITOLS

by

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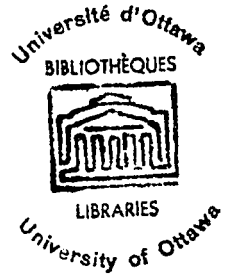
A thesis submitted in partial fulfillment
of the requirements of the degree of
Doctor of Philosophy



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PREFACE

This thesis is the result of work that was undertaken in order to make a contribution to the synthetic chemistry of aminocyclitols. This class of compounds commands widespread interest inasmuch as, in recent years, several representatives of it have been discovered as components of important antibiotics. In the Introduction, the significance of aminocyclitols in the context of antibiotics chemistry will be outlined, and the methods available for their synthesis will be reviewed. In the main part of the thesis is described, first, the synthesis and characterization of several hitherto unknown aminocyclitols which, it is hoped, may be of value in future efforts at structural elucidation of new antibiotics. Secondly, a number of synthetic approaches to 2-deoxystreptamine are described which, although having fallen short of ultimate success, may prove useful as basis for later projects.

The candidate wishes to express his deepest gratitude to Professor Hans H. Baer, for his stimulating guidance, his valuable instructions and especially his patience and encouragement throughout the whole process of this research.

Kind suggestions made by Dr. T. Neilson and Dr. M.C. Cook are gratefully acknowledged.

The candidate is obliged to the Department of Chemistry, University of Ottawa, and also to many friends for making this research possible.

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ABSTRACT

Part I.

The catalytic hydrogenation of 4,6-dinitropyrogallol was investigated under a variety of conditions. Whereas it proved difficult to hydrogenate the benzene ring with palladium catalysts, the use of platinum catalyst led to an uptake of nine moles of hydrogen as required for the production of diamino-trihydroxycyclohexanes. The hydrogenation products were isolated as solid hydrochlorides or sulfates in yields of 29-46%. They were shown by paper chromatography to be mixtures of several compounds. Ion exchange column chromatography resulted in the isolation, as crystalline dihydrochlorides, of five unknown aminocyclitols:— three stereoisomeric diamino-trihydroxy-cyclohexanes and two stereoisomeric diamino-dihydroxycyclohexanes. The compounds were characterized by their chromatographic mobilities relative to 2-deoxystreptamine (R_{ds} 0.74, 0.80, 0.96, 1.15, and 1.35), by elemental analyses, x-ray diagrams, and by the preparation of derivatives. Their structures and configurations followed from periodate oxidation and nuclear magnetic resonance studies. The following assignments were made.

Compound R_{ds} 0.74: 1α,3α-diamino-4α,5α,6α,trihydroxy-cyclohexane (XV)

Compound R_{ds} 0.80: D,L-1α,3α-diamino-4α,5α,6β-trihydroxycyclohexane (XVI)

Compound R_{ds} 0.96: D,L-1α,3β-diamino-4α,5α,6α-trihydroxycyclohexane (XVII)

Compound R_{ds} 1.15: 1α,3α-diamino-4α,6α-dihydroxy-cyclohexane (XVIII)

Compound R_{ds} 1.35: D,L-1α,3α-diamino-4α,6β-dihydroxycyclohexane (XIX)

Part II.

For a stereospecific synthesis of 2-deoxystreptamine (XI) the diketone XXVII, 4,5,6-xylo-trimethoxy-1,3-cyclohexanedione, was needed. It was attempted to synthesize this diketone in a number of ways. Reaction of 2,3,4-tri-O-methyl xylaryl chloride (XXXVI) and methylene dimagnesium diiodide or methylene cadmium did not yield the expected product. A Dieckmann type cyclization of dimethyl 2,3,4-tri-O-methyl-xylarate (XXXII) with methyl (or ethyl) acetate failed to produce the expected 4,5,6-xylo-trimethoxy-2-carboalkoxy-1,3-cyclohexanedione (XXXIX). Methyl hydrogen 2,3,4-tri-O-methyl-D,L-xylarate (XLI) was prepared from the corresponding diester XXXII by partial hydrolysis. The half ester XLI was converted to an acyl chloride, γ-carbomethoxy-D,L-xylo-trimethoxybutyryl chloride (XLIII). This acyl chloride was reacted with diazomethane followed by hydriodic acid reduction of the diazoketone, in the expectation that methyl 6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XL) ($C_{10}H_{18}O_6$) be formed. However, the reaction product appeared to have the composition $C_{12}H_{20}O_9$. Reaction of the acyl chloride XLIII and dimethyl cadmium gave a compound $C_{10}H_{20}O_6$.

INTRODUCTION

1. On Antibiotics

The word "antibiotic" was first used by Waksman (1) to refer to "chemical substances, that are produced by microorganisms, and that have the capacity to inhibit and even to destroy other microorganisms." Benedict and Langlykke (2) suggested that the definition should include inhibitory substances of plant or animal origin. In a recent review, Vonderbank (3) proposed that an antibiotic be defined as a compound possessing inhibitory, i.e., static, degenerative, lyzing, or killing activities for vegetable or animal microorganisms such as viruses, rickettsia, bacteria, actinomycetes, fungi, algae or protozoa. Such a broader definition takes into account a number of more recent developments in the field. To date, more than 600 antibiotics have been discovered in nature. Although the majority of them are of limited medical value, some 30 have found clinical application, and many of these have proved extremely beneficial in the combat of a great variety of infectious diseases. From a chemical point of view, antibiotics are not easily classified because of the great variety of structures that are encountered. However, many of the more

important antibiotics may be grouped together on the basis of their structures being made up of, or containing, carbohydrate moieties. Prominent among the carbohydrate units found in these compounds are branched-chain sugars, amino sugars, and aminocyclitols. As a background for the experimental work undertaken for this thesis, some aspects of the chemistry of those antibiotics which contain aminocyclitols (Tab.I) will be reviewed in the following sections.

2. Aminocyclitols as Constituents of Antibiotics

A. Nomenclature of the Aminocyclitols

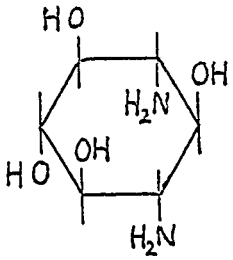
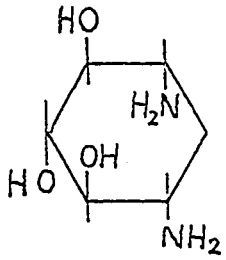
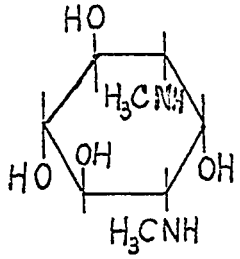
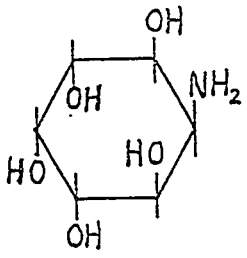
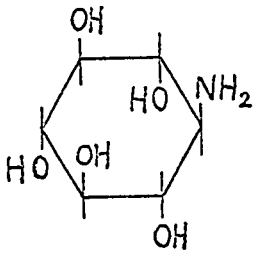
The term "aminocyclitols" is used for the general description of polyfunctional amino alcohols derived from cyclohexane. Thus, amino-deoxy derivatives of inositols (cyclohexanehexols), quercitols (cyclohexanepentols), and cyclohexanetetrols are being referred to by this term. Monoamino-monodeoxy-inositols have been called "inosamines", and diamino-dideoxy-inositols, "inosadiamines". Although three different systems of rational nomenclature for inositol derivatives have been devised (4,5,6), the trivial names of the compounds listed in Table I are used almost exclusively throughout the literature.

B. Streptamine in the Streptomycins

The antibiotic, streptomycin (7), was obtained in 1944 from cultures of Streptomyces griseus by Waksman, Schatz and Bugie (8). The substance has a low toxicity and is active against Gram-positive and Gram-negative organisms. It is used in the treatment of many infections which are resistant to penicillin, such as tularemia (9), typhoid fever (10), tuberculosis (11), and brucellosis (12). Dihydrostreptomycin, mannosidostreptomycin, and hydroxystreptomycin are closely

Table I

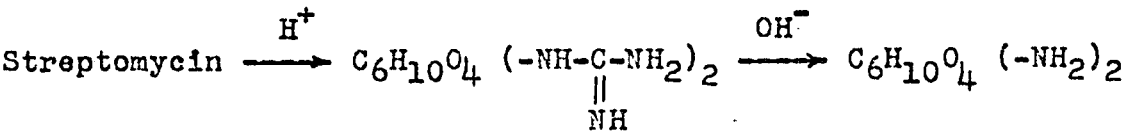
Aminocyclitols Found in Antibiotics

Aminocyclitol	Structure	Antibiotics	References
Streptamine		streptomycin	15, 17
		dihydrostreptomycin	
		mannosidostreptomycin	
		hydroxystreptomycin	
2-Deoxystreptamine		neomycins	33
		paromomycins	47
		kanamycins	37
		gentamycins	48
		hygromycin B	50
Actinamine		actinospectacin	60
neo-Inosamine-2		hygromycin	64, 69
		antibiotic 1703-18B	67
scyllo-Inosamine		bluensomycin	71

related to streptomycin; in particular, they contain the same aminocyclitol moiety.

The elucidation of the structure of streptomycin began in 1946 and led to the formula shown in Fig. 1 by the efforts of research groups at Merck and Company, the Squibb Institute for Medical Research, the Ohio State University and the University of Illinois. The work included degradation and partial synthesis, and although the correct formula (Fig.1) was elaborated by 1950, confirmation by nuclear magnetic resonance studies of certain configurational details was advanced as late as 1962, and the synthesis of one constituent, streptose, was reported only in 1965 (13). A total chemical synthesis of streptomycin has not been accomplished as yet.

Acid hydrolysis of streptomycin gave a nitrogen-rich degradation product that was called streptidine and was characterized in the form of several crystalline salts (14,15,16). Alkaline hydrolysis of streptidine produced a diamino compound that was named streptamine, and further investigations revealed that streptidine was the corresponding diguanidino derivative.



Streptidine Streptamine

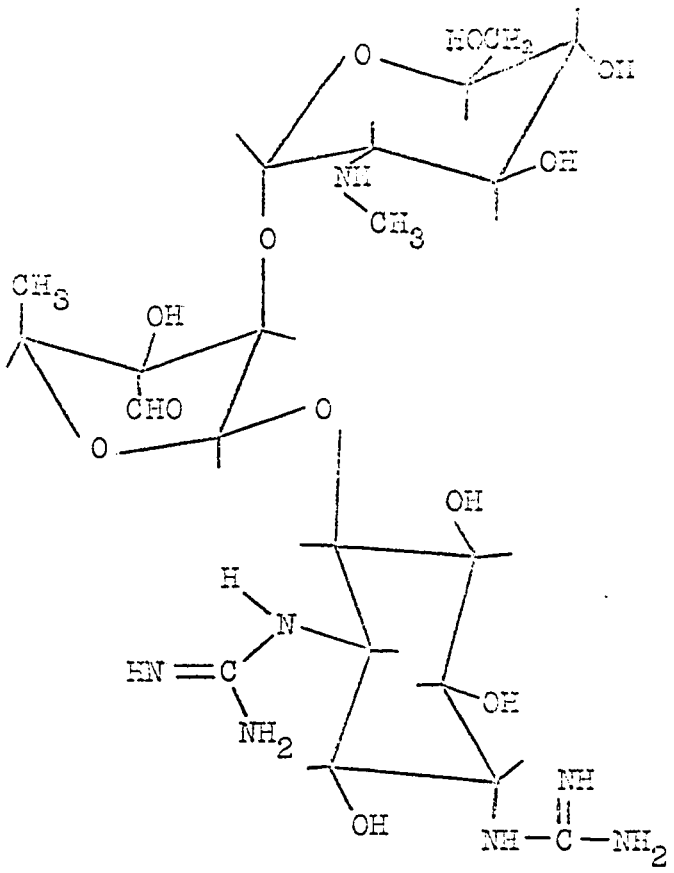
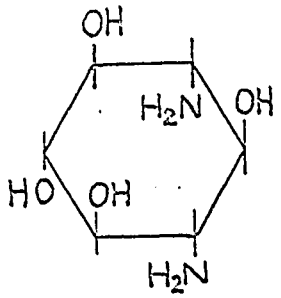


Fig. 1. Streptomycin

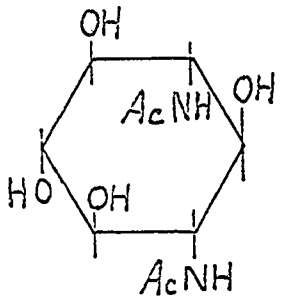
The structure of streptamine (I) was established as follows. Folkers (17) and Carter (15) found that the compound having composition $C_6H_{14}N_2O_4$ contains two amino and four hydroxyl groups, and consumes six moles of periodate to give two moles of ammonia but no formaldehyde; thus the hexa-substituted cyclohexane structure was established. They also demonstrated that N,N'-diacetylstreptamine (II) consumed only two moles of periodate. This fact indicated that the acetamido groups in II are at 1,3- or 1,4-positions with respect to each other; 1,2-position would have required an uptake of three moles of periodate. Periodate cleavage followed by bromine oxidation of N,N'-dibenzoylstreptamine (III) led to dibenzamido-hydroxyglutaric acid (IV) (18). When hexaacetylstreptamine (V) was heated in a sealed tube at 350° for one hour, two crystalline products were obtained (17). One of them was identified as 2,4-diacetamidophenol (VI) by comparison with a sample prepared by catalytic hydrogenation of 2,4-dinitrophenol in acetic anhydride-acetic acid. The second product was shown to be 5-acetamido-2-methylbenzoxazole (VII) by acid hydrolysis to 2,4-diaminophenol dihydrochloride. These facts proved streptamine to be a 1,3-diamino-2,4,5,6-tetrahydroxycyclohexane.

Since streptamine is optically inactive, the two amino groups in positions 1 and 3 must be <u>cis</u> to each other, and the two hydroxyl groups at positions 4 and 6 must also be <u>cis</u> to each other. Further stereochemical features of the molecule were established by Wintersteiner and Klingsberg (19). These



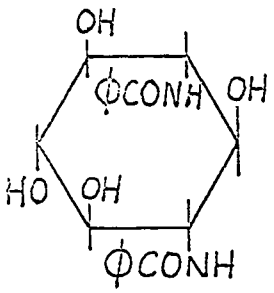
I

$\xrightarrow[6\ moles]{NaIO_4}$ $2\ NH_3$, no HCHO



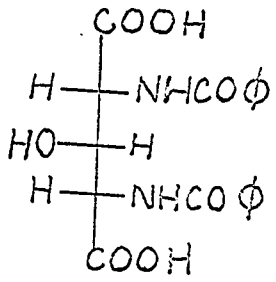
II

$\xrightarrow[2\ moles]{NaIO_4}$ dialdehyde

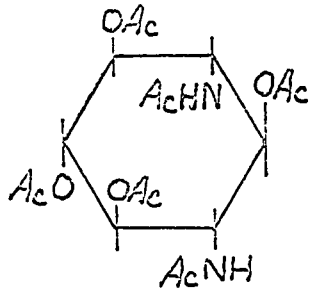


III

$\xrightarrow{NaIO_4}$ $\xrightarrow{Br_2 - H_2O}$

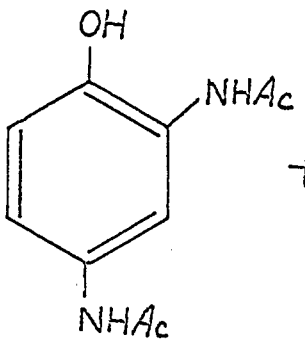


IV



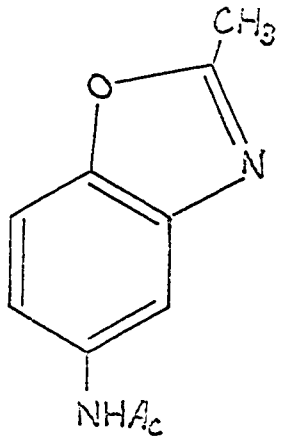
V

$\xrightarrow{350^\circ}$



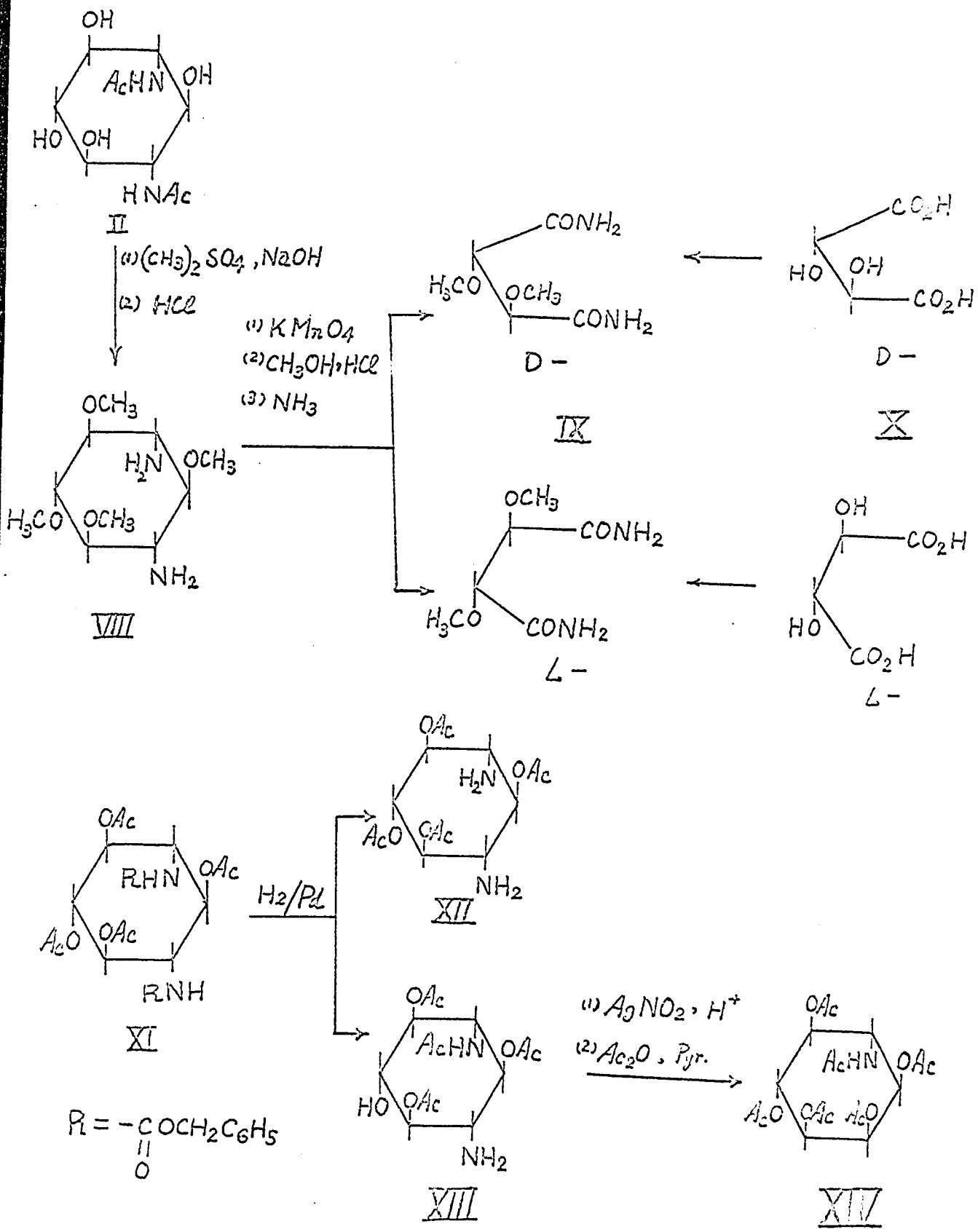
VI

+



VII

workers obtained tetra-O-methylstreptamine (VIII) by O-methylation and subsequent hydrolysis of the N,N'-diacetyl derivative II. The methyl ether VIII was oxidized with potassium permanganate to give a racemic mixture of acids that were characterized as their crystalline amides following esterification and ammonolysis. The amides were found to be identical with D- and L-dimethoxysuccinamide (IX) obtained from D,L-tartaric acid (X). This experiment established that in streptamine the three hydroxyl groups at the positions 4,5, and 6 are all-*trans* with respect to one another, since an all-*cis* configuration would have given *meso*-dimethoxysuccinic acid. The configuration of the hydroxyl group at position 2 was also established by Wintersteiner and Klingsberg (19). N,N'-Dicarbobenzoxy-tetra-O-acetylstreptamine (XI) was catalytically reduced with palladium to yield, besides the expected tetra-O-acetylstreptamine (XII), an isomeric N-acetyl-tri-O-acetyl-streptamine (XIII) that resulted from O $\longrightarrow$ N migration of one acetyl group from position 5 to position 3. Compound XIII was deaminated with silver nitrite in acidic medium, then acetylated to yield hexaacetyl-D,L-*myo*-inosamine-4 (XIV) whose stereochemistry had been previously elucidated (20). Since, in XIV, the acetamido group and its flanking acetoxyl groups form a *trans*-*trans* system, it follows that in I the hydroxyl group in 2-position must be *trans* to the two amino groups. Hence, streptamine has all-*trans* configuration. Confirmation of this came from nuclear magnetic


II
(1) $(CH_3)_2SO_4$, NaOH
(2) HCl
(1) $KMnO_4$
(2) CH_3OH, HCl
(3) NH_3
VIII
IX
X
D-
L-
XI
H_2/Pd
XII
XIII
XIV
(1) $AgNO_2$, H^+
(2) Ac_2O, Pyr.
$R = -C(=O)OCH_2C_6H_5$

resonance studies on N,N,N',N'-tetramethylstreptamine (21) and on hexaacetylstreptamine (22). Streptamine was ultimately synthesized by Wolfrom and coworkers (23); the details are discussed in Section 3.

C. 2-Deoxystreptamine

(1) 2-Deoxystreptamine in the Neomycins

The antibiotic, neomycin, was discovered by Waksman and Lechevalier (24) in 1949. It was found active against Gram-positive, Gram-negative, and acid-fast bacteria, including streptomycin-resistant strains of tubercle bacilli. The antimicrobial activity is comparable to that of streptomycin (25).

Countercurrent distribution studies by Waksman and coworkers (26) revealed that the antibiotic consisted of at least three active components subsequently designated as neomycins A,B, and C. These active components were isolated by Folkers et al. (27), Regna et al. (28), and Wintersteiner et al. (29), respectively. The compound originally described as neomycin A (also called neamine) was recognized later to be a fragment of both neomycin B and C rather than a true neomycin (30). The structure of the neomycins was determined chiefly by Rinehart (31).

Neomycin C is composed of two 2,6-diamino-2,6-dideoxy-D-glucose molecules, D-ribose, and 2-deoxystreptamine (Fig.2). In neomycin B, a molecule of 2,6-diamino-2,6-dideoxy-L-idose is substituted for one of the two diaminoglucoses. The two antibiotics are, therefore, identical except for opposite

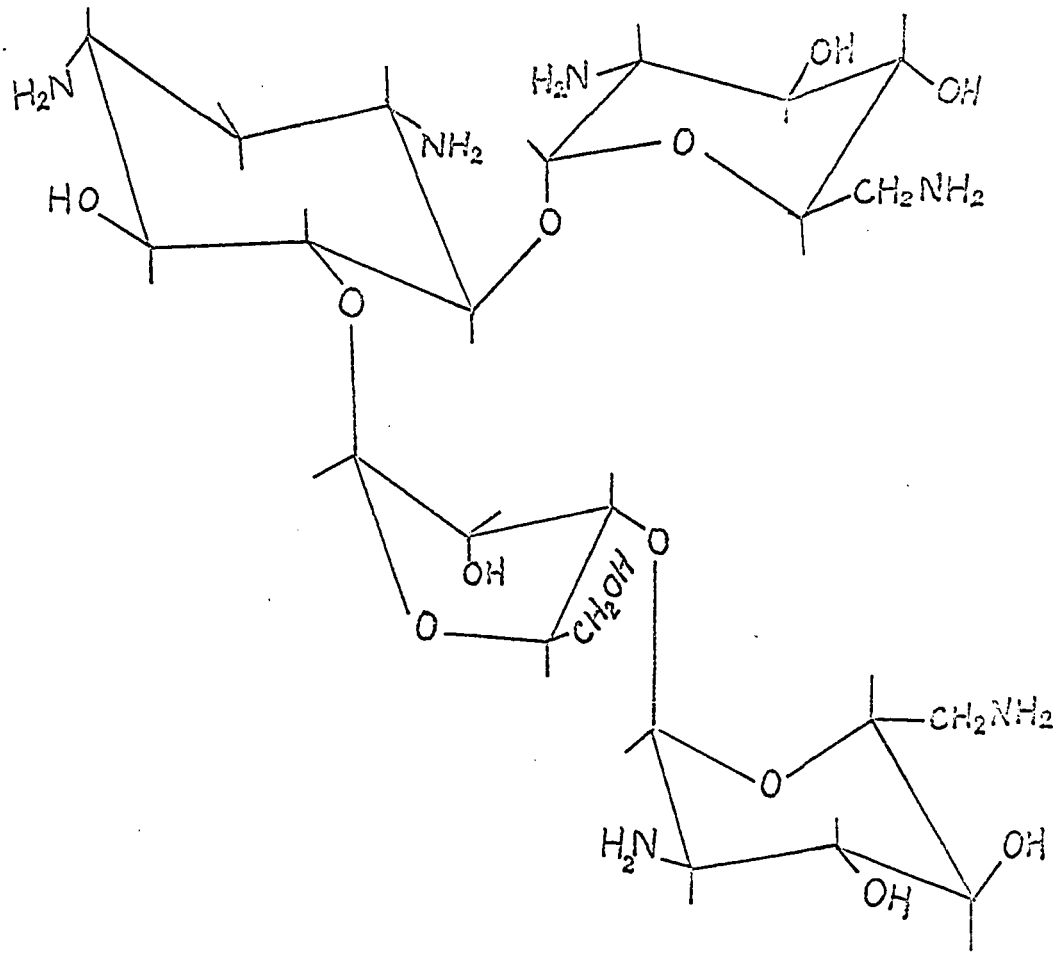
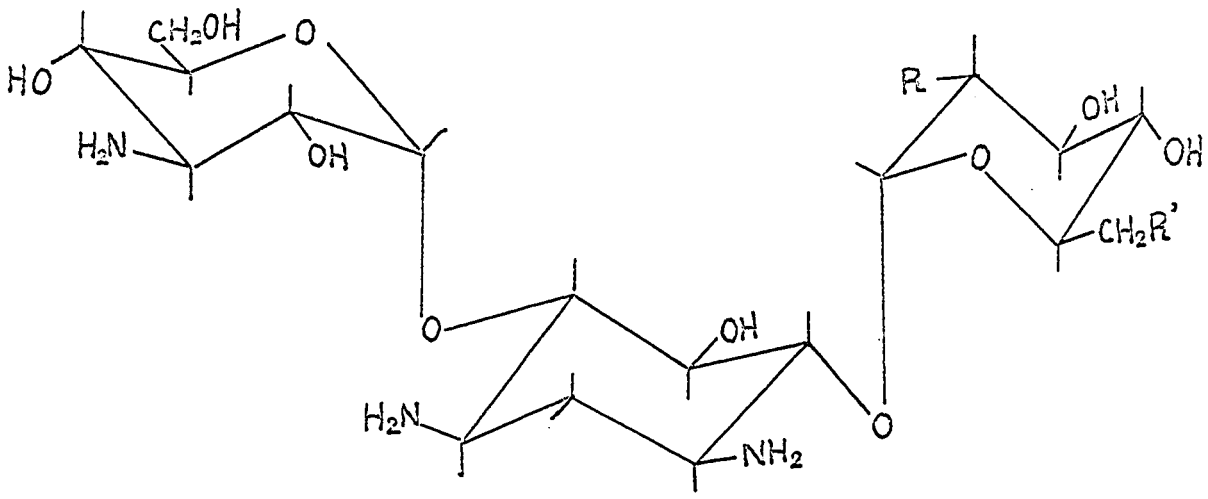


Fig.2. Neomycin C.

configurations at a single asymmetric carbon atom. Mild methanolysis of either antibiotic leads to neamine, a fragment consisting of 2-deoxystreptamine and 2,6-diamino-2,6-dideoxy-D-glucose (29,32). This fragment is cleaved into its components by strong acid hydrolysis (33).

(2). 2-Deoxystreptamine in the Kanamycins

An antibiotic named kanamycin was first isolated from cultures of Streptomyces kanamyceticus by Umezawa in Japan (34). It shows a fairly low toxicity and is very active against a variety of Gram-positive and Gram-negative bacteria including streptomycin-resistant strains. Crude kanamycin was separated into a major component (kanamycin A) and a minor component (kanamycin B) (35). A closely related substance, kanamycin C, was isolated and studied recently (36). The three antibiotics possess a common structural pattern that was largely elucidated by Lemieux at the University of Ottawa and in collaboration with Bristol Laboratories and, independently, by Umezawa and coworkers in Japan. The three kanamycins are each composed of three components, two of which they have in common, namely 2-deoxystreptamine and 3-amino-3-deoxy-D-glucose. They differ in the third component which is 6-amino-6-deoxy-D-glucose, 2-amino-2-deoxy-D-glucose, or 2,6-diamino-2,6-dideoxy-D-glucose (Fig.3). The infrared spectrum of 2-deoxystreptamine dihydrobromide obtained from kanamycin (37) was identical with that of the product isolated from the neomycins (30).



Kanamycin A, R=-OH, R'=-NH_2

Kanamycin B, R=R'=-NH_2

Kanamycin C, R=-NH_2, R'=-OH

Fig. 3. Configurations of kanamycins A, B, and C.

(3). 2-Deoxystreptamine in the Paromomycins

Paromomycin was obtained from culture filtrates of a strain of streptomyces (38). It possesses a high degree of effectiveness in the treatment of intestinal amebiasis (39). A number of other antibiotics — catenulin (40), hydroxymycin (41), aminosidine (42) and zygomycin (43) — have been revealed to be identical with paromomycin (44,45). Besides the main antibiotic (now paromomycin I) there was isolated recently an isomer, paromomycin II (45,46). The structure of the paromomycins is depicted in Figure 4 (31,47). It is seen that the paromomycins are related to the neomycins on the one hand, and to the kanamycins on the other. The aminocyclitol from paromomycin was identified (47) as 2-deoxystreptamine.

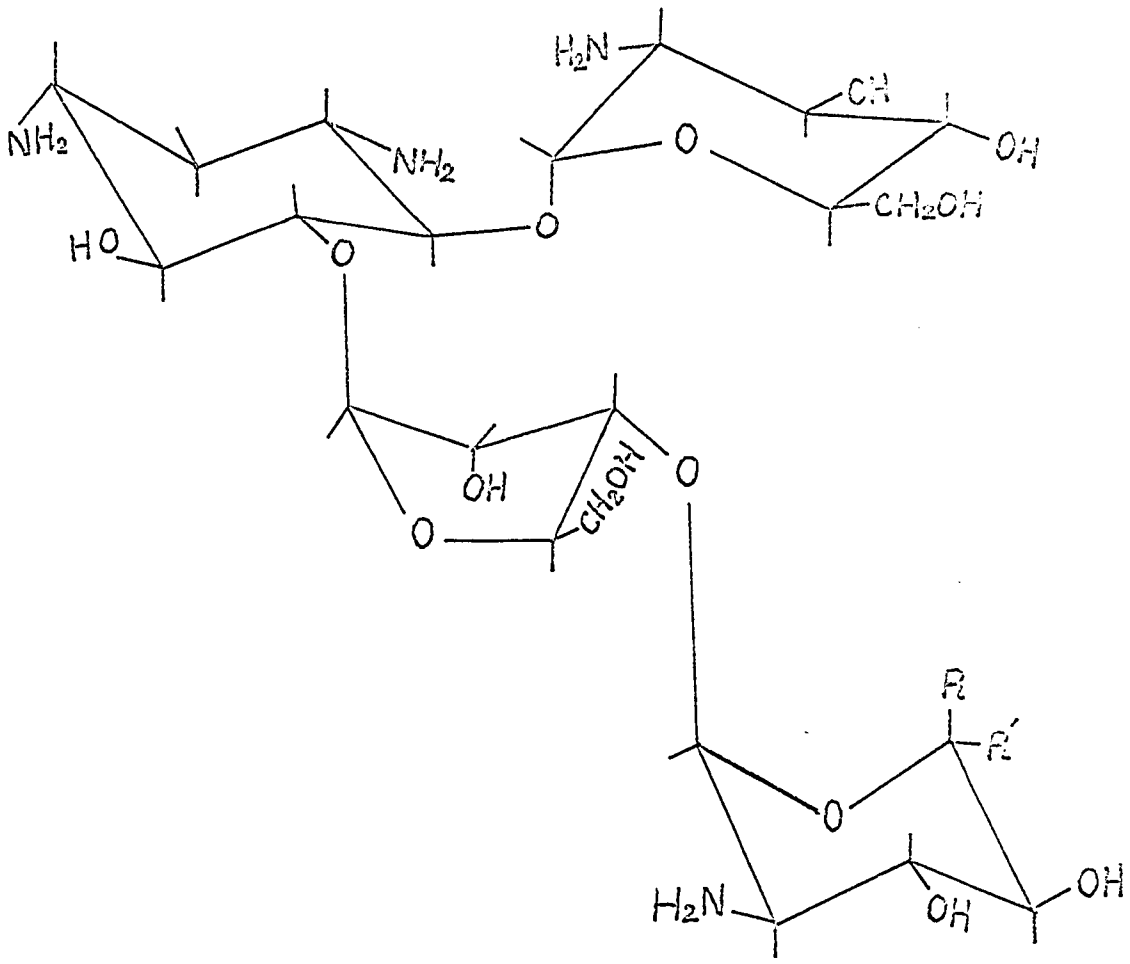
(4). 2-Deoxystreptamine in Gentamycins C_1 and C_2

Recently, Weinstein and coworkers (48) described a fourth family of 2-deoxystreptamine antibiotics, the gentamycins. They were isolated from Micromonospora species, which are microorganisms that differ from the streptomycetes. The gentamycins C_1 and C_2 (which apparently are isomeric) gave 2-deoxystreptamine on acid hydrolysis.

(5). Derivatives of 2-Deoxystreptamine in Antibiotics

(a) N-Methyl-2-deoxystreptamine in Hygromycin B

The antibiotic, hygromycin B (49), was shown by Wiley, Sigal and Weaver (50) to give on acid hydrolysis a new aminocyclitol. The product, which was called hyosamine, contained



Paromomycin I, R=-CH_2NH_2, R'=H

Paromomycin II, R=H, R'=-CH_2NH_2

Fig. 4. Configurations of paromomycins I and II.

an amino and a methylamino group. Hyosamine dihydrochloride consumed four moles of periodate, and N,N',N'-trimethyl-hyosamine dihydrochloride was found to be identical with N,N, N',N'-tetra-methyl-2-deoxystreptamine dihydrochloride (51). Consequently, hyosamine must be N-methyl-2-deoxystreptamine (50).

(b). N-Acetyl-2-deoxystreptamine in Neomycins LP_b and LP_c

Recently, Chilton and Rinehart (52,53) have shown two members of the neomycin family, the neomycins LP-I and LP-II, to be mono-N-acetylated neomycins B and C, respectively, and they have renamed them neomycins LP_b and LP_c, accordingly. In either isomer the N-acetyl group is situated in the 2-deoxystreptamine moiety. This was established by isolation of the N'-methyl-2-deoxystreptamine from the N-acetylneamine by reductive N'-methylation of the latter compound and subsequent hydrolysis of the methylated product; the acetamido group in the 2-deoxystreptamine moiety appeared as the free amino group in the final product.

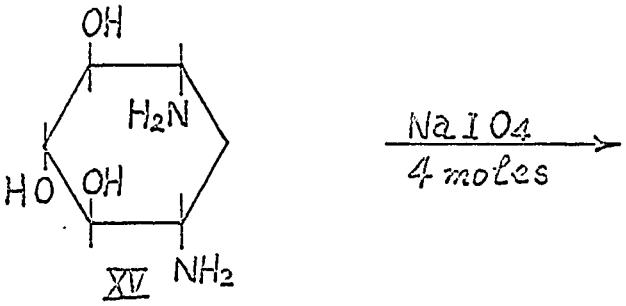
Neomycin LP_b ⟶ N-Acetylneamine

Neomycin LP_c ⟶ N-Acetylneamine

N-Acetylneamine —N'-methylation→ N-Acetyl-N'-methylneamine —hydrolysis→ N'-Methyl-2-deoxystreptamine.

(6). Structure Elucidation of 2-Deoxystreptamine

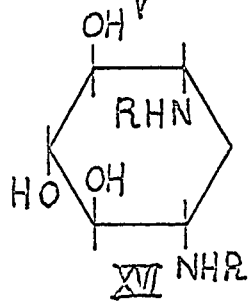
As early as 1951, Kuehl, Bishop and Folkers (33) demonstrated that 2-deoxystreptamine (XV) obtained from neomycin consumed four moles of periodate. It was thereby established that all hydroxyl and amino groups were vicinal. N,N'-Dibenzoyl-2-deoxystreptamine (XVI), on the other hand, consumed only two moles of periodate, which indicated that the three hydroxyl groups must be vicinal to one another. The dialdehyde XVII obtained in the latter oxidation was converted into a dithioacetal, XVIII, that was reduced over Raney nickel to give meso-2,4-dibenzamidopentane (XIX). A reference sample of XIX was synthesized (54) starting from acetylacetone. Thus, 2-deoxystreptamine was shown to be a 1,3-diamino-4,5,6-trihydroxy-cyclohexane. The workers (33) also suggested that the configuration of this aminocyclitol was all-trans, by analogy to that of streptamine. Since 2-deoxystreptamine is optically inactive, the two amino groups must be cis to each other, and the two hydroxyl groups in the 4- and 6-positions must be cis to each other as well. Carter and Dyer (55) proposed that the amino groups were trans to the neighboring hydroxyl groups, since the dibenzoyl derivative XVI did not undergo N $\longrightarrow$ O benzoyl migration in mild acid, under conditions known to effect such migration in cis-, but not in trans-2-benzamidocyclohexanol (56). The three contiguous hydroxyl groups were assigned trans-trans configuration on the basis of the rate of periodate uptake by the



XV

$NaIO_4$ / 4 moles →

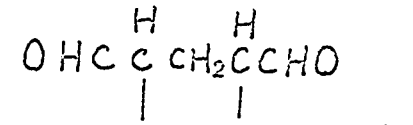
(1) C_6H_5COCl, Pyr.
(2) $Ba(OCH_3)_2$, CH_3OH ↓



XVI

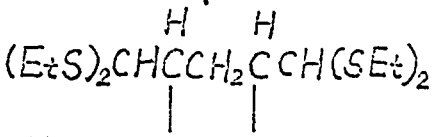
$R = -COC_6H_5$

$NaIO_4$ / 2 moles →



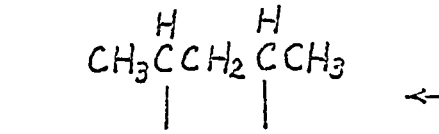
XVII

EtSH, HCl ↓



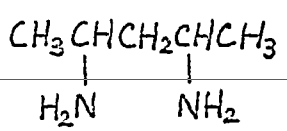
XVIII

← Raney Ni, H_2

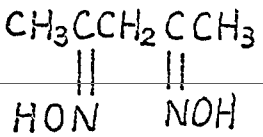


XIX

↑ (1) C_6H_5COCl
(2) separation of *meso*- and racemic forms



← [H]



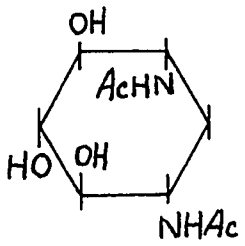
↑ NH_2OH

$CH_3COCH_2COCH_3$
acetylacetone

dibenzoyl derivative XVI, which was slow and nearly identical to that of N,N'-dibenzoylstreptamine (III) (55). A faster rate would have been expected, had a cis-glycol grouping been present. The all-trans configuration of the three hydroxyl groups was also supported by electrophoretic studies (57). N,N'-Diacetyl-2-deoxystreptamine (XX) shows complete lack of migration in borate buffer and thus behaves like (the all-trans) scyllo-inositol, whereas myo-inositol (containing one axial hydroxyl) migrates at considerable speed, and inositols with 2 or 3 axial hydroxyls move even more rapidly.

In 1963, Lemieux and Cushley (58) recorded n. m. r. spectra of 2-deoxystreptamine and of some derivatives. By analysis of the ring hydrogen signals they were able to prove conclusively the all-equatorial arrangement of the substituents, thus confirming the evidence previously invoked for the configuration.

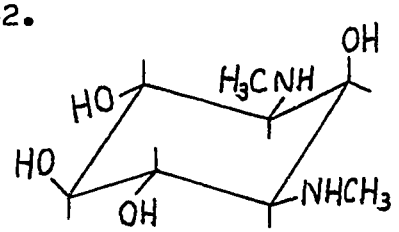
2-Deoxystreptamine was finally synthesized by Nakajima, Hasegawa and Kurihara (59) in 1964. The synthesis involved eighteen steps starting from benzene. The details will be discussed in Section 3.



XX

D. Actinamine in Actinospectacin

Wiley (60) recently isolated a base called actinamine from the hydrolysis products of a new antibiotic, actinospectacin, which originated from Streptomyces spectabilis (61). Actinamine was found to be an inositol derivative bearing two methylamino groups. The elucidation of its structure and configuration, which was done largely by n.m.r. spectroscopy (62), led to formula XXI. As can be seen, the compound differs from streptamine, apart from N,N'-dimethylation, only in the configuration at C-2.



XXI

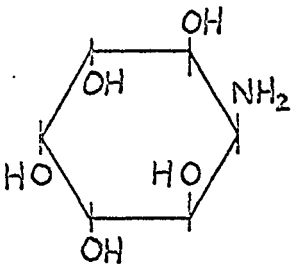
E. neo-Inosamine-2 in Antibiotics

Hygromycin is an antibiotic having activity against Gram-positive and Gram-negative bacteria and actinomycetes (63). It was extracted from fermentation broths of Streptomyces hygroscopicus. The main structural features of hygromycin were established by Mann and Woolf (64). When hygromycin was refluxed in hydrochloric acid, an inosamine hydrochloride was isolated. The amine and its hexaacetyl and N-acetyl derivatives showed no optical activity. On deamination with nitrous acid the

inosamine yielded myo-inositol (XXII). Posternak (65) had shown that the deaminations of both myo-inosamine-2 (XXIII) and scyllo-inosamine (XXIV) take place with Walden inversion leading to the formation of scyllo-inositol (XXV) and myo-inositol (XXII), respectively. Assuming that Walden inversion also occurred in the case of the hygromycin component, Mann and Woolf (64) suggested that the formation of XXII would imply formula XXIV or XXVI for their compound. Only these two of the eight possible, optically inactive inosamines could yield XXII by this mechanism. The infrared spectrum and the x-ray diffraction pattern of the inosamine differed from those of an authentic sample of XXIV (66). Therefore, the compound was tentatively identified as the heretofore unknown neo-inosamine-2 (XXVI).

Patrick and coworkers (67) reported the isolation of an antibiotic provisionally called 1703-18 B. Upon acid hydrolysis, this antibiotic also gave neo-inosamine-2 (XXVI). It appears that the antibiotic 1703-18 B is similar to, though not identical with, hygromycin.

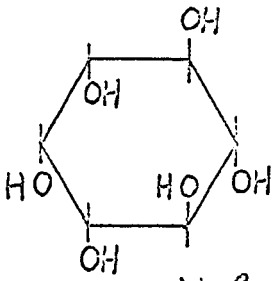
Homomycin, an antibiotic isolated by Sumiki and coworkers (68) in Japan, was reported to be identical (69) with hygromycin.



neo - inosamine-2

XXVI

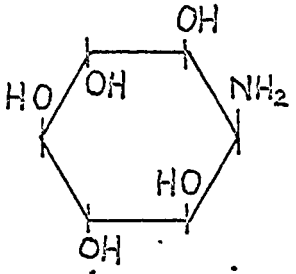
HNO_2



myo - inositol

XXII

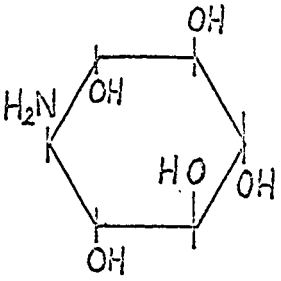
HNO_2



myo - inosamine-2

XXIII

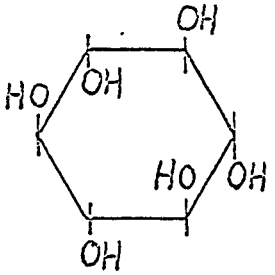
HNO_2



scyllo - inosamine

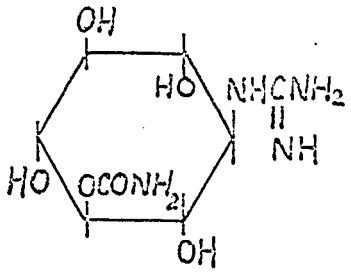
XXIV

HNO_2



scyllo - inositol

XXV



XXVIII

F. scyllo-Inosamine in Bluensomycin

The isolation of a new basic antibiotic, bluensomycin, has been described recently by Bergy and coworkers (70). Structural studies on this antibiotic were undertaken by Bannister and Argoudelis (71) who obtained a degradation product, bluensidine, on mild methanolysis at room temperature. Hydrolysis of bluensidine under reflux with saturated barium hydroxide gave two moles of carbon dioxide, three moles of ammonia, and an optically inactive aminocyclitol. This aminocyclitol consumed six moles of periodate and was found to be identical with scyllo-inosamine (XXIV), by comparison of the hexaacetyl and the N-acetyl derivatives with authentic samples (72). Bluensidine is 1-guanidino-3-O-carbamoyl-scyllo-inositol (XXVII).

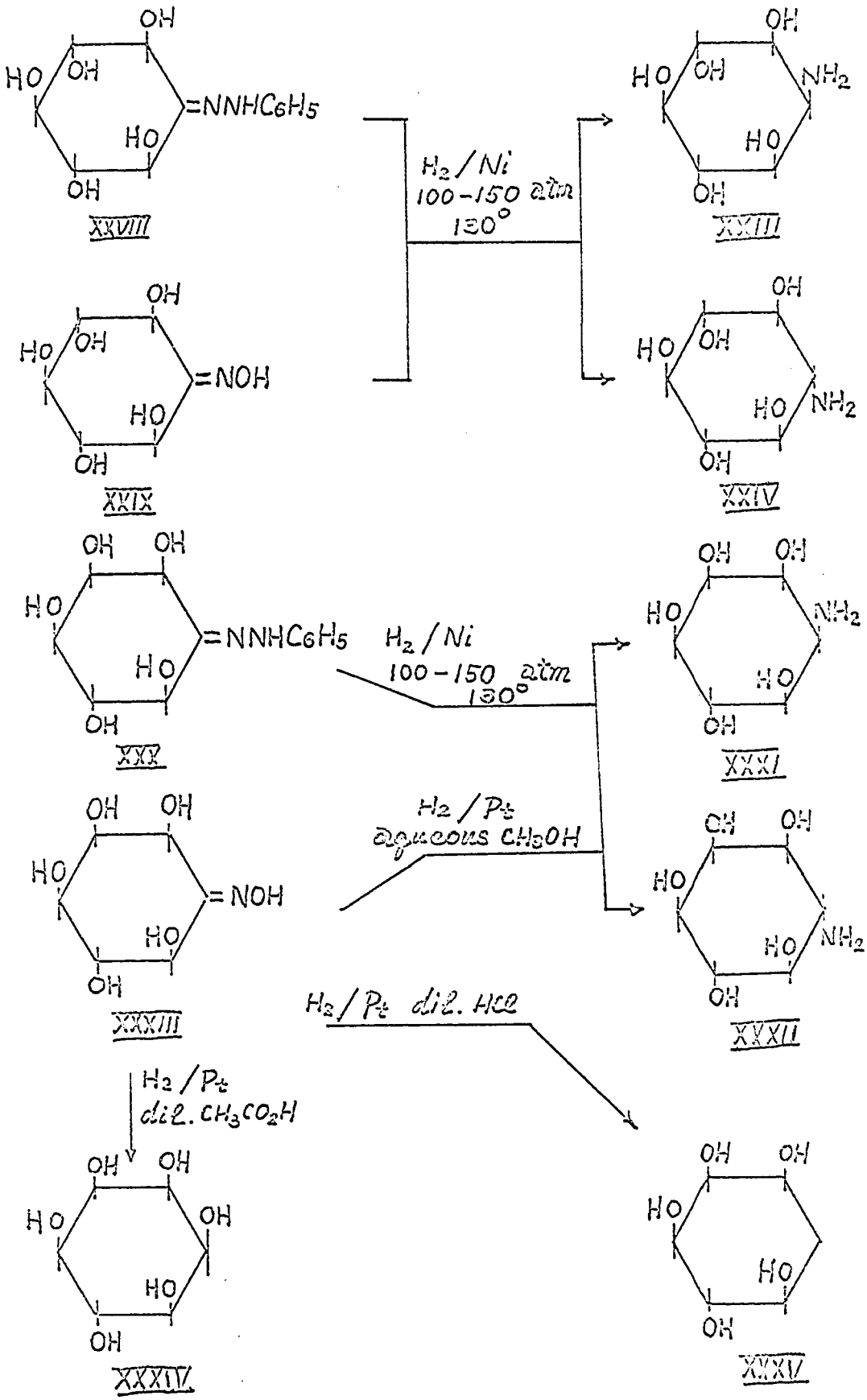
3. Methods for the Synthesis of Aminocyclitols

The methods described below have been employed successfully in partial and total syntheses of aminocyclitols that are either constituents of antibiotics or hitherto unknown compounds.

A. Reduction of Oximes or Phenylhydrazones

Reductions of >C=N< bonds in phenylhydrazones and oximes of keto-deoxy-inositols have been employed extensively in partial syntheses of inosamines, and less frequently in those of inosadiamines. Carter and coworkers (73) investigated the catalytic reduction, with Raney nickel, of the phenylhydrazone (XXVIII) and the oxime (XXIX) of keto-deoxy-scyllo-inositol (74,79) and of the phenylhydrazone (XXX) of DL-2-keto-2-deoxy-epi-inositol (75). The phenylhydrazone XXVIII and the oxime XXIX gave the same mixture of myo-inosamine-2 (XXIII) and scyllo-inosamine (XXIV). Under the same conditions the phenylhydrazone XXX yielded only one of the two possible epimers, XXXI or XXXII.

May and Mosettig (76) reported that the hydrogenation products of the oxime (XXXIII) of DL-2-keto-2-deoxy-epi-inositol varied with the reaction conditions employed, Thus, catalytic hydrogenation of XXXIII with platinum oxide in 70% methanol gave one of two epimers, XXXI or XXXII, in a yield of 70%. When the hydrogenation was conducted in dilute acetic acid, a mixture consisting of epi-inositol (XXXIV) (yield, 25%) and a small amount of XXXI (or XXXII), was obtained. When the


XXVIII
XXIX
XXIII
XXIV
XXX
XXXI
XXXIII
XXXII
XXXIV
XXXV
H_2 / Ni
100 – 150 atm
130°
H_2 / Pt
aqueous CH_3OH
H_2 / Pt dil. HCl
H_2 / Pt
dil. CH_3CO_2H

catalytic hydrogenation was done in dilute hydrochloric acid, no nitrogenous products but only a deoxyinositol (XXXV) and a very small amount of epi-inositol (XXXIV) were formed. Anderson and Lardy (72) showed that the phenylhydrazone XXVIII and the oxime XXIX gave almost exclusively a single isomer, XXIII (or XXIV) in high yields when platinum oxide in glacial acetic acid was used. However, when the oxime XXIX was reduced with sodium amalgam, scyllo-inosamine (XXIV) was produced, and isolated as its hexaacetyl derivative, in a yield of 73%.

Latham, May and Mosettig (78) also investigated the catalytic hydrogenation of the oxime XXIX. Using platinum oxide in 50% aqueous methanol they obtained only one product, myo-inosamine-2 (XXIII), in a yield of 55%.

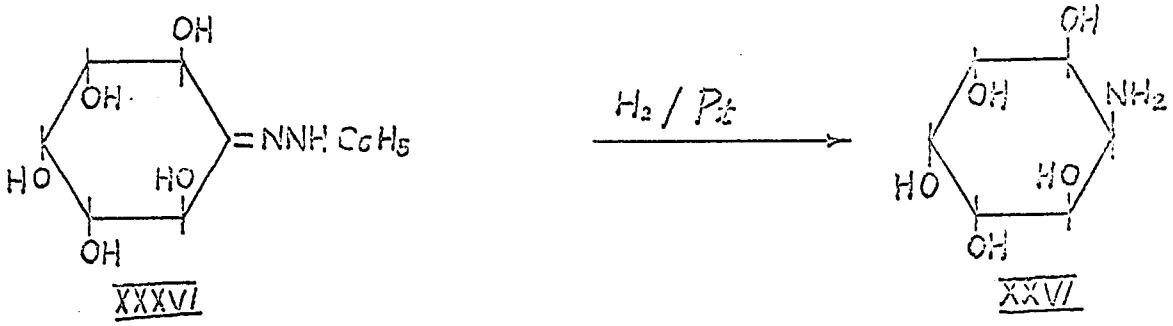
The configuration of the inosamine (XXXI or XXXII) which had been prepared by Carter et al. (73) from the phenylhydrazone XXX and by May and Mosettig (76) from the oxime XXXIII was not established until Straube-Rieke and coworkers (20) obtained, by sodium amalgam reduction of the same oxime XXXIII, a second inosamine. They were then able to assign, to their new product the configuration of DL-myo-inosamine-4 (XXXII), and to the previous product, the configuration of D,L-epi-inosamine-2 (XXXI).

Allen (80) performed the synthesis of neo-inosamine-2 (XXVI), a component of the antibiotic, hygromycin; the compound was obtained in a yield of 53% by platinum-catalyzed hydrogenation of the phenylhydrazone XXXVI.

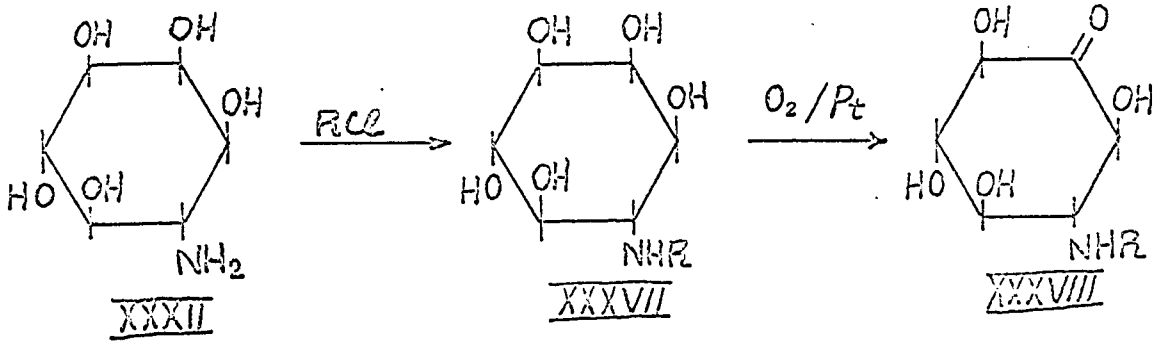
The steric direction of an amino group formed by reduction of an oxime or a phenylhydrazone is specifically influenced by the reducing agent used and the conditions employed. The experiments cited above show that catalytic reduction tends to direct the new group into cis position with respect to its neighboring substituents, whereas sodium amalgam reduction favors the trans configuration.

The first synthesis of an inosadiamine by catalytic hydrogenation of an oxime was reported by Heyns and Paulsen (81). These workers converted D,L-myo-inosamine-4 (XXXII) into its N-carbobenzoxy derivative XXXVII, the axial hydroxy group of which was then oxidized in the presence of a platinum catalyst (82) to give N-carbobenzoxy-D,L-2-keto-myo-inosamine-4 (XXXVIII). The oxime XXXIX of this ketone was reduced with sodium amalgam to give streptamine (I), that was isolated as its N,N'-dicarbobenzoxy derivative XL, and was shown to be identical with the natural product (15,17).

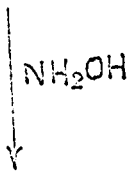
Recently, Japanese workers (59) announced the total synthesis of 2-deoxystreptamine (XV). Their route included eighteen steps starting from benzene. Benzene was first photochlorinated (83) in the presence of iodine to yield 3,4,5,6-tetrachlorocyclohexene-1 (XLI) which was hydroxylated to cis-1,2-dihydroxy-3,4,5,6-tetrachlorocyclohexane (XLII). Dechlorination of this product followed by acetylation gave cis-1,2-diacetoxy-cyclohexadiene (XLIII). The diene XLIII

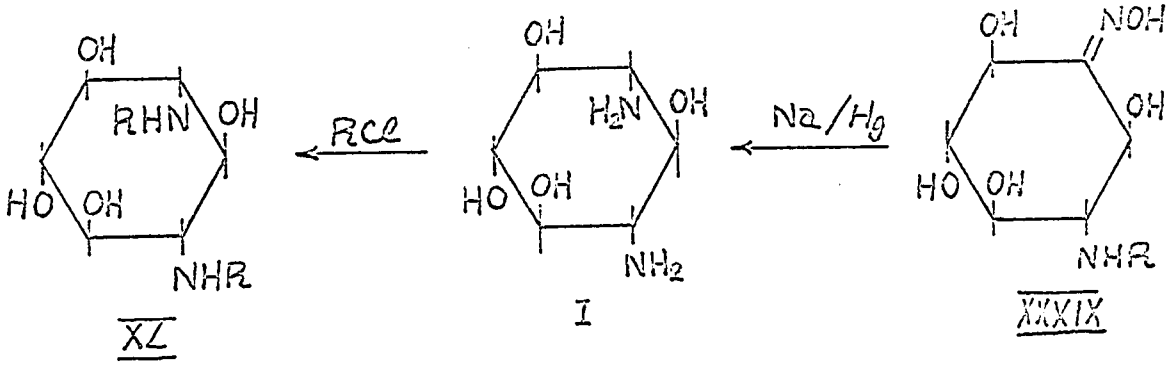


XXXVI XXV



XXXII XXXVII XXXVIII

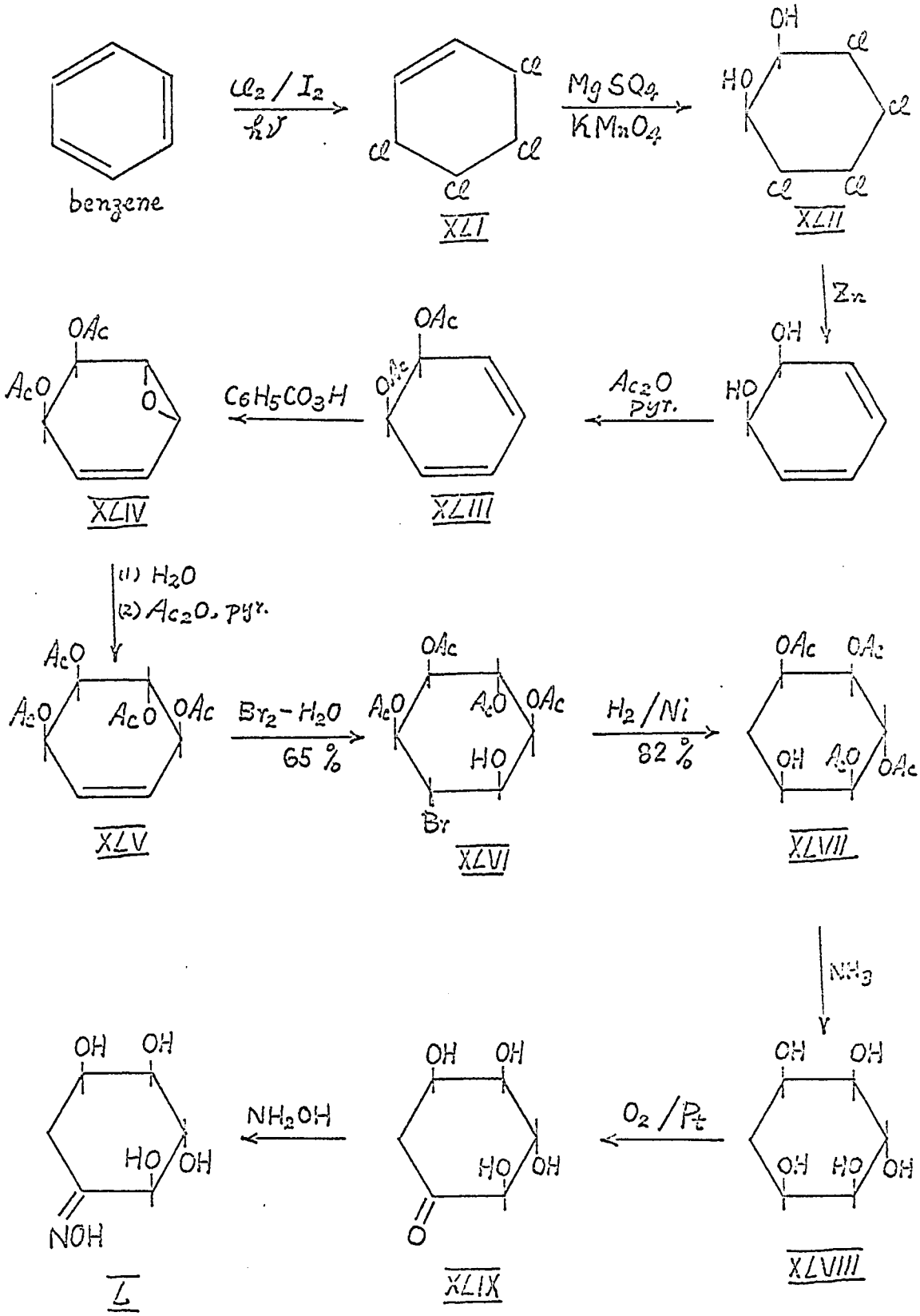


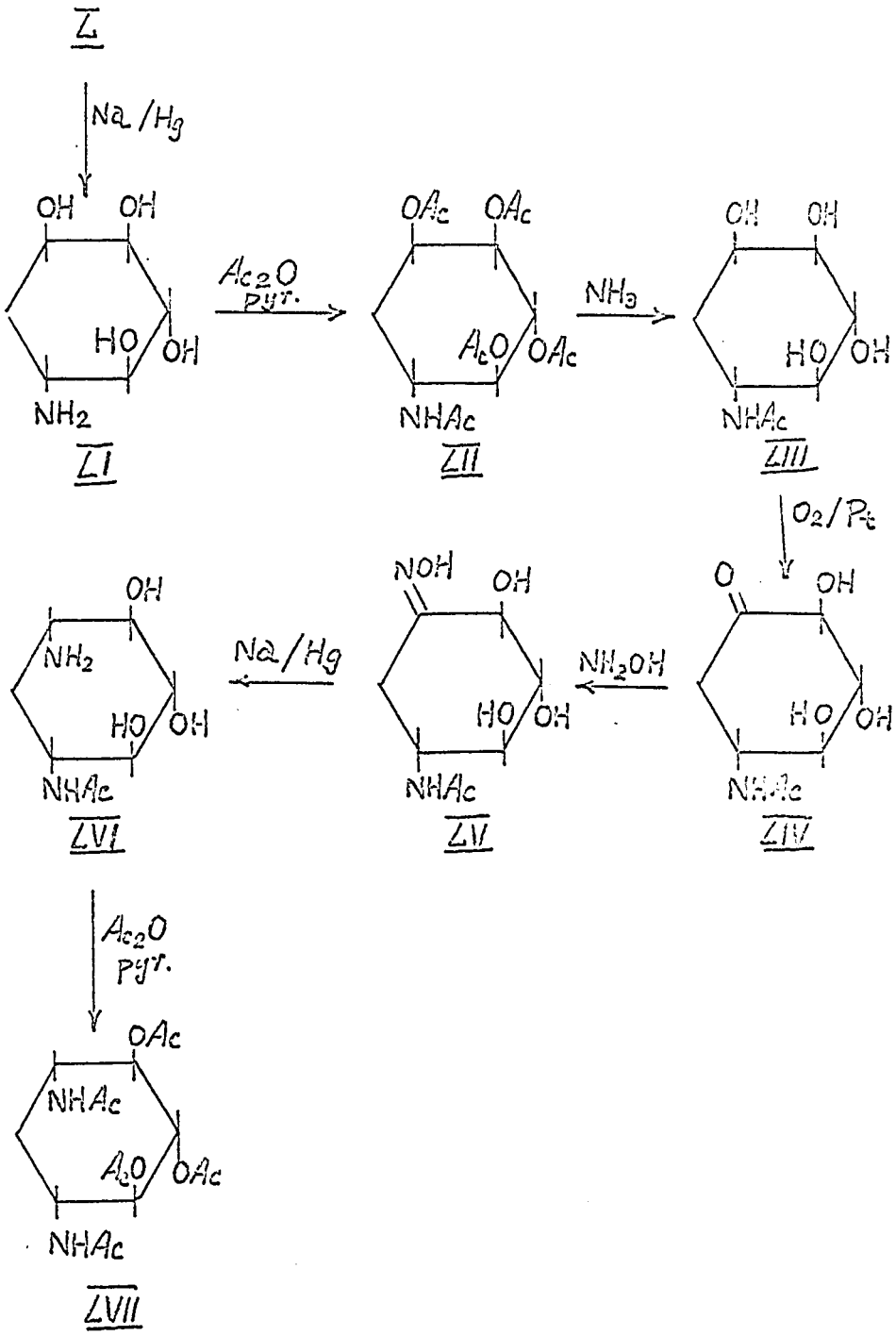


XL I XXXIX

$$R = -\underset{\underset{O}{\|}}{C}OCH_2C_6H_5$$

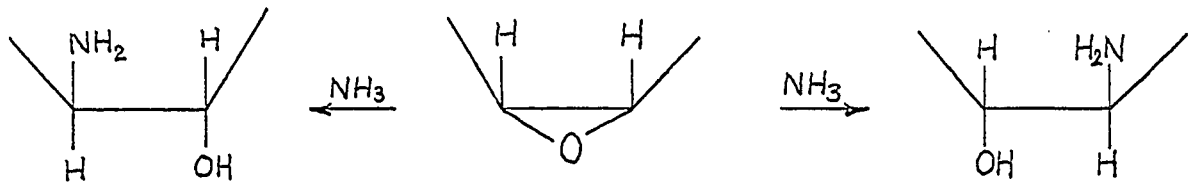
was treated with peroxybenzoic acid to give an epoxydiacetate XLIV (84) which, upon hydrolysis and acetylation, afforded the tetraacetate XLV (85). This tetraacetate was converted into the bromohydrin XLVI, D,L-3-bromo-3-deoxy-1,2,5,6-tetraacetyl-muco-inositol, in a yield of 65%. The isolation of only one isomer was explained by preferential diaxial addition of hypobromous acid (86). Hydrogenolysis of XLVI with Raney nickel gave D,L-3-deoxy-1,2,5,6-tetraacetyl-epi-inositol (XLVII). At this point, the introduction of the deoxy function required for 2-deoxystreptamine had been achieved. Ammonolysis to 3-deoxy-epi-inositol (XLVIII) followed by catalytic oxidation (87) furnished D,L-2-keto-2,3-dideoxy-epi-inositol (XLIX). The oxime L of this ketone was reduced with sodium amalgam at pH 6-6.5 to give D,L-3-deoxy-myo-inosamine-4 (LI) which was converted into its N-acetate LIII via the pentaacetate LII. Catalytic oxidation of the N-acetate LIII led to the ketone LIV whose oxime, LV, was reduced with sodium amalgam to yield N-acetyl-2-deoxystreptamine (LVI). Finally, pentaacetyl-2-deoxystreptamine (LVII) was prepared and was found to be identical with an authentic sample obtained from kanamycin (37).


benzene
Cl_2 / I_2
$h\nu$
XLI
$MgSO_4$
$KMnO_4$
XLII
Zn
XLIII
Ac_2O
Pyr.
$C_6H_5CO_3H$
XLIV
(1) H_2O
(2) Ac_2O, pyr.
XLV
$Br_2 - H_2O$
65 %
XLVI
H_2 / Ni
82 %
XLVII
NH_3
XLVIII
O_2 / Pt
XLIX
NH_2OH
L


Z
Na/Hg
OH
OH
HO
OH
NH2
ZI
Ac2O
Pyr.
OAc
OAc
AcO
OAc
NHAc
ZII
NH3
OH
OH
HO
OH
NHAc
ZIII
O2/Pt
O
OH
HO
OH
NHAc
ZIV
NH2OH
NOH
OH
HO
OH
NHAc
ZV
Na/Hg
OH
NH2
HO
OH
NHAc
ZVI
Ac2O
Pyr.
OAc
NHAc
AcO
OAc
NHAc
ZVII

B. Ammonolysis of Epoxides and Halohydrins

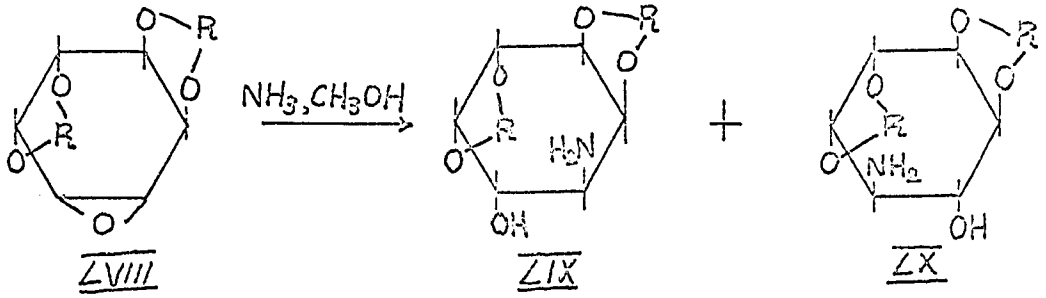
Cyclitol epoxides may react with ammonia to give two isomeric aminocyclitols according to the following scheme.



The hydroxyl and amino groups formed are trans-related, and the proportion of the isomers depends on the structure and stereochemistry of the parent cyclitol epoxide.

Cyclitol epoxides (1,2-anhydrocyclitols) are readily prepared by two general methods: (a), the action of bases on tosyl derivatives in which an adjacent hydroxyl group is trans-situated, and (b), treatment of cyclic olefin derivatives with peracids. The former method has been used by Angyal and coworkers(77,88),the latter by Nakajima and coworkers (89).

Employing the above principles, Allen (90) reported a synthesis of two new inosamines. When L-1,2-anhydro-3,4; 5,6-di-O-isopropylidene-allo-inositol (LVIII) was treated with ammoniacal methanol at 100°, a mixture of L-2,3;4,5-di-O-isopropylidene-neo-inosamine-1 (LIX) and 1,2;3,4-di-O-isopropylidene-L-inosamine-5 (LX) was obtained. Upon

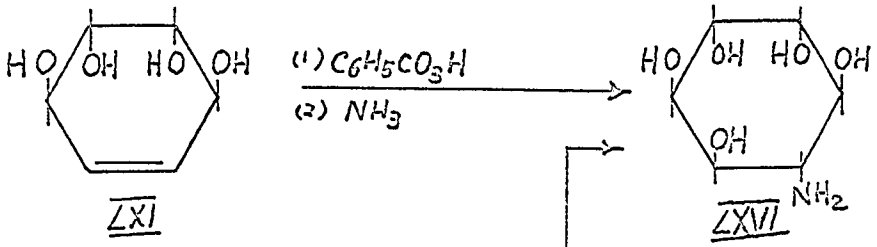


LVIII

LIX

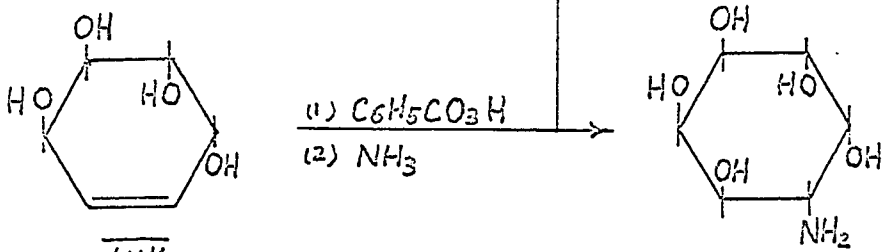
LX

R = $-C(CH_3)_2-$



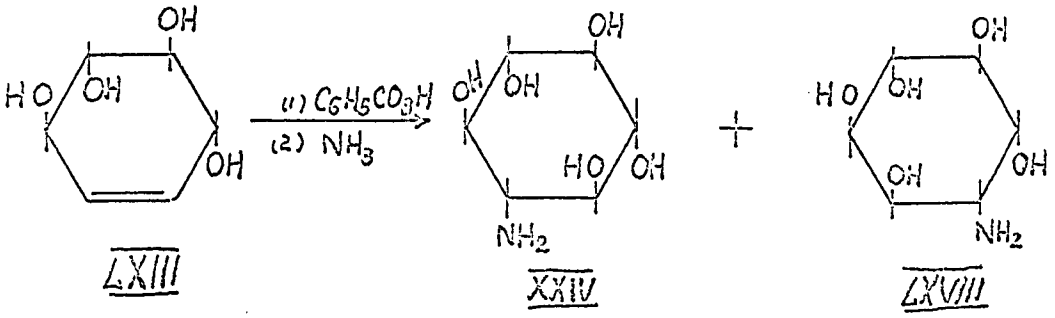
LXI

LXVI



LXII

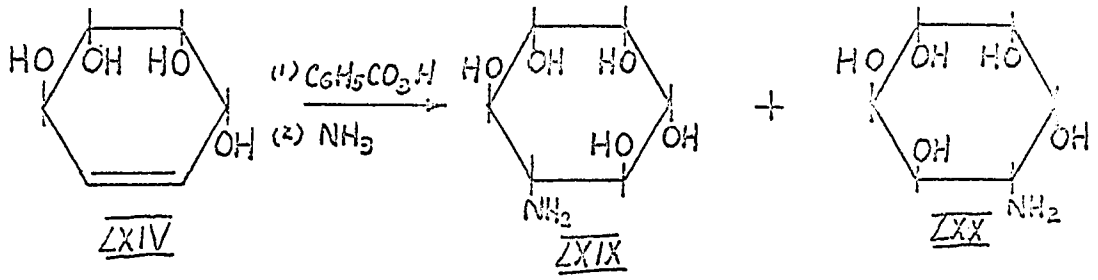
LXVII



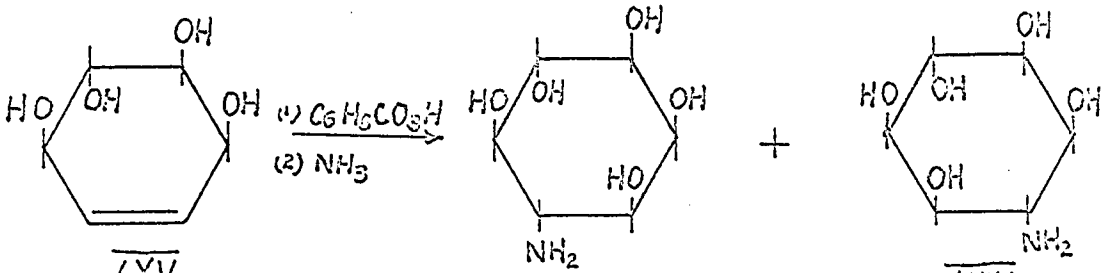
LXIII

LXIV

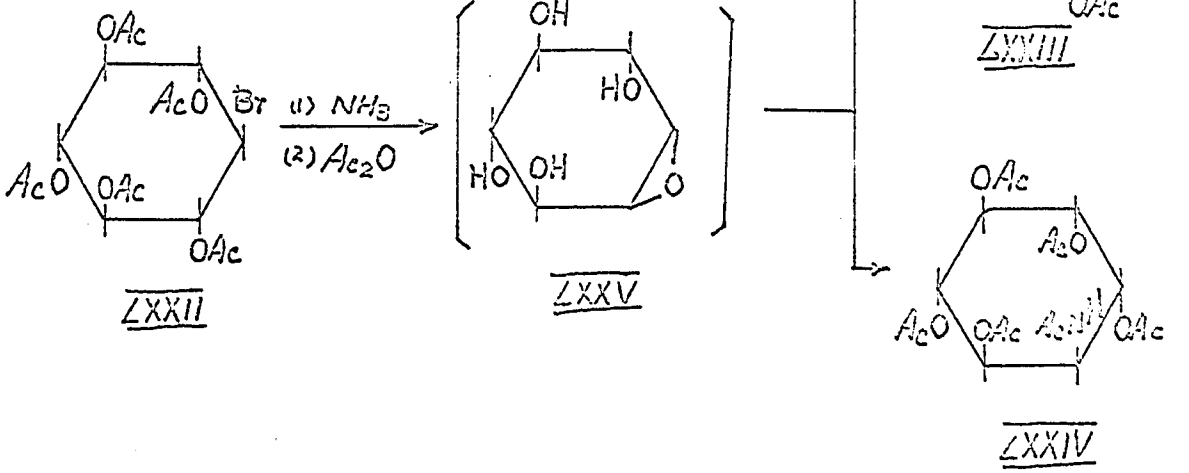
LXVIII



LXIV LXIX LXX



LXV XXXII LXXI

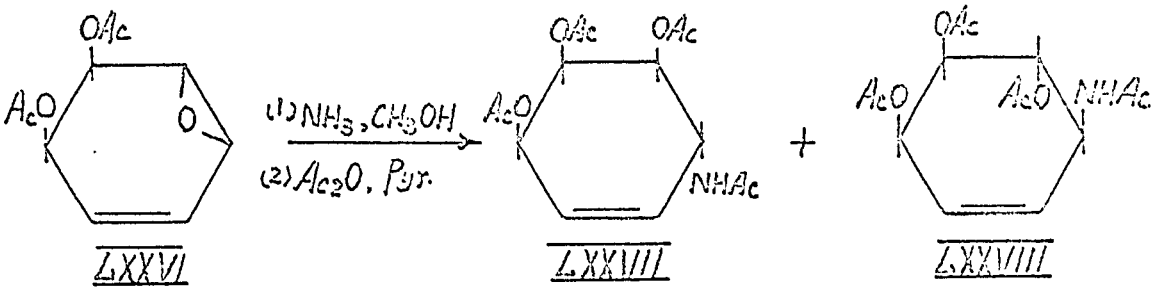


LXXII LXXV LXXIII LXXIV

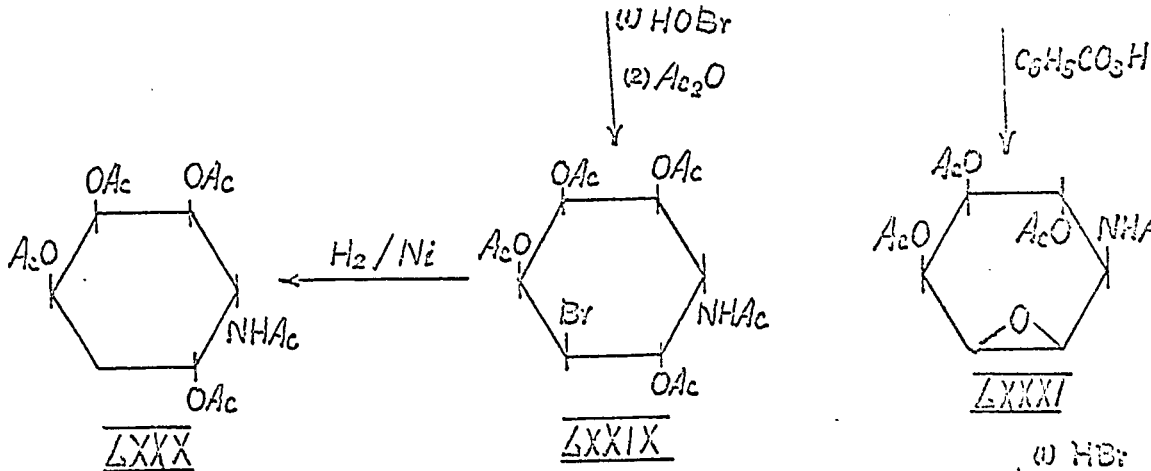
removal of the isopropylidene groups the inosamines were separated by fractional crystallization and characterized by N-acetylation.

Starting from conduritols (tetrahydroxycyclohexenes), Nakajima, Kurihara and Hasegawa (91) recently synthesized eight different inosamines, three of which had been unknown. The conduritols LXI, LXII, LXIII, LXIV and LXV (92) were first converted into the epoxides. The latter compounds were then treated with ammoniacal methanol and the products were acetylated without prior isolation. Fractional crystallizations then afforded the eight inosamines, of which LXVI, LXVII (90), XXIV (73), XXXII (76) and LXVIII (93) had been isolated and characterized previously. The new inosamines isolated were: myo-inosamine-5 (LXIX), allo-inosamine-1 (LXX) and muco-inosamine-3 (LXXI).

Alternative starting materials for aminocyclitol syntheses are bromohydrins of the inositol series. Thus, Wolfrom and coworkers (93) treated pentaacetyl-bromodeoxy-scyllo-inositol (LXXII) with ammonia and obtained, in poor yields after reacetylation, hexaacetyl-scyllo-inosamine (LXXIII) and hexaacetyl-D,L-inosamine-2 (LXXIV). The reaction presumably proceeded via the epoxide intermediate (LXXV). Similarly, a number of inosadiamines have been obtained from dibromo-dideoxy-inositols, but no structural or configurational assignments have been made as yet (93,94).



LXXVI LXXVIII LXXVIII



LXXX LXXIX LXXXI

LXXXII

LXXXIII

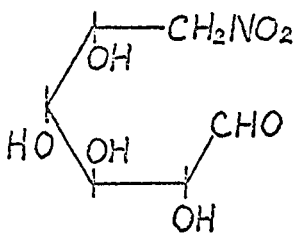
LXXXIV

The principle of introducing an amino group by ammonolysis of epoxides and the principle of reducing a bromohydrin to generate a deoxy function (cf. the synthesis of 2-deoxystreptamine, p.28) were combined by Nakajima et al. (95) in recent work that furnished several deoxyinosamines. The diacetoxy-epoxy-cyclohexene LXXVI, for instance, gave upon ammonolysis and subsequent acetylation the two stereoisomeric triacetoxy-acetamido-cyclohexenes, LXXVII and LXXVIII. Addition to LXXVII of hypobromous acid followed by acetylation afforded the bromo compound LXXIX which was debrominated with Raney nickel to give pentaacetyl D,L-5-amino-1,5-dideoxy-allo-inositol (LXXX). The cyclohexene LXXVIII was converted with peroxybenzoic acid into the epoxide LXXXI from which pentaacetyl D,L-1-amino-1,3-dideoxy-epi-inositol (LXXXII) was obtained by successive reaction with hydrobromic acid, acetylation, and reductive debromination. Similar reaction sequences starting from stereoisomers of LXXVI led to the pentaacetates of D,L-1-amino-1,3-dideoxy-myo-inositol (LXXXIII) and D,L-1-amino-1,3-dideoxy-allo-inositol (LXXXIV).

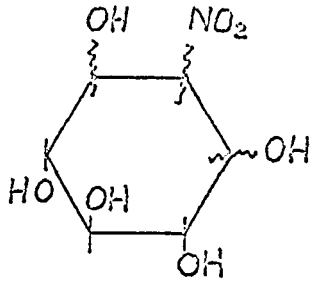
C. Syntheses Involving Nitroalkane Cyclization

A new principle for the synthesis of nitrogenous inositol derivatives was elaborated by H.O.L. Fischer and his associates. When a 6-deoxy-6-nitro-aldose is exposed to mild alkali, it cyclizes to a mixture of stereoisomeric deoxy-nitroinositols, e.g., LXXXV ⟶ LXXXVI. In this reaction, two

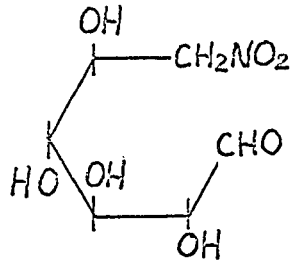
new asymmetric centers are generated. Moreover, epimerizations will occur at the carbon atoms adjacent to the nitromethyl and nitromethylene groups. Consequently, a total of 8 stereoisomers can theoretically arise. In practice, however, this number is reduced to two or three because of conformational preferences. Thus, Grosheintz and Fischer (96) obtained three deoxynitroinositols upon cyclization of 6-deoxy-6-nitro-D-glucose (LXXXV), and the same products arose from 6-deoxy-6-nitro-L-idose (XC), the 5-epimer of LXXXV. The configurations of the products were not determined at the time, nor was reduction to the amino stage one of the main objectives of the work, although such reduction was shown to be feasible. Later, the configurations were established (97) to be scyllo (LXXXVII), myo-1 (LXXXVIII) and muco-3 (LXXXIX) (the latter arising by kinetic control and disappearing in thermodynamic equilibrium), so that the corresponding inosamines are now potentially available through this route. The starting compounds for the above cyclizations, 6-deoxy-6-nitro-D-glucose (LXXXV) and -L-idose (XC), were obtained (98) by condensation with nitromethane of 1,2-isopropylidene-D-xylodialdose (XCI) followed by acid removal of the isopropylidene blocking group. It has subsequently been shown that free xylodialdose (xylo-trihydroxyglutaric dialdehyde) can be cyclized with nitromethane, directly and without isolation of intermediates, to yield the scyllo-and myo-1-deoxy-nitroinositols LXXXVII and LXXXVIII (99).



LXXXV

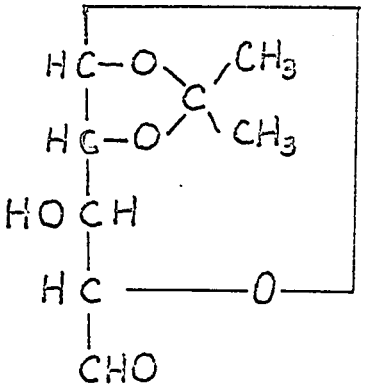


LXXXVI

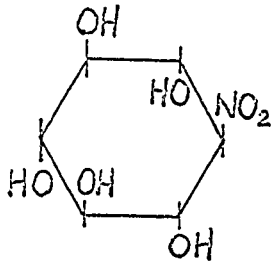


XC

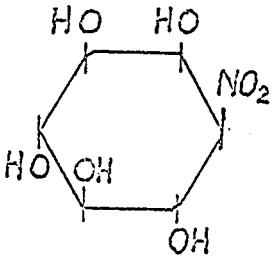
CH_3NO_2



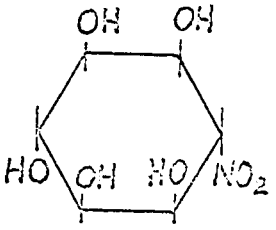
XCI



LXXXVII



LXXXVIII



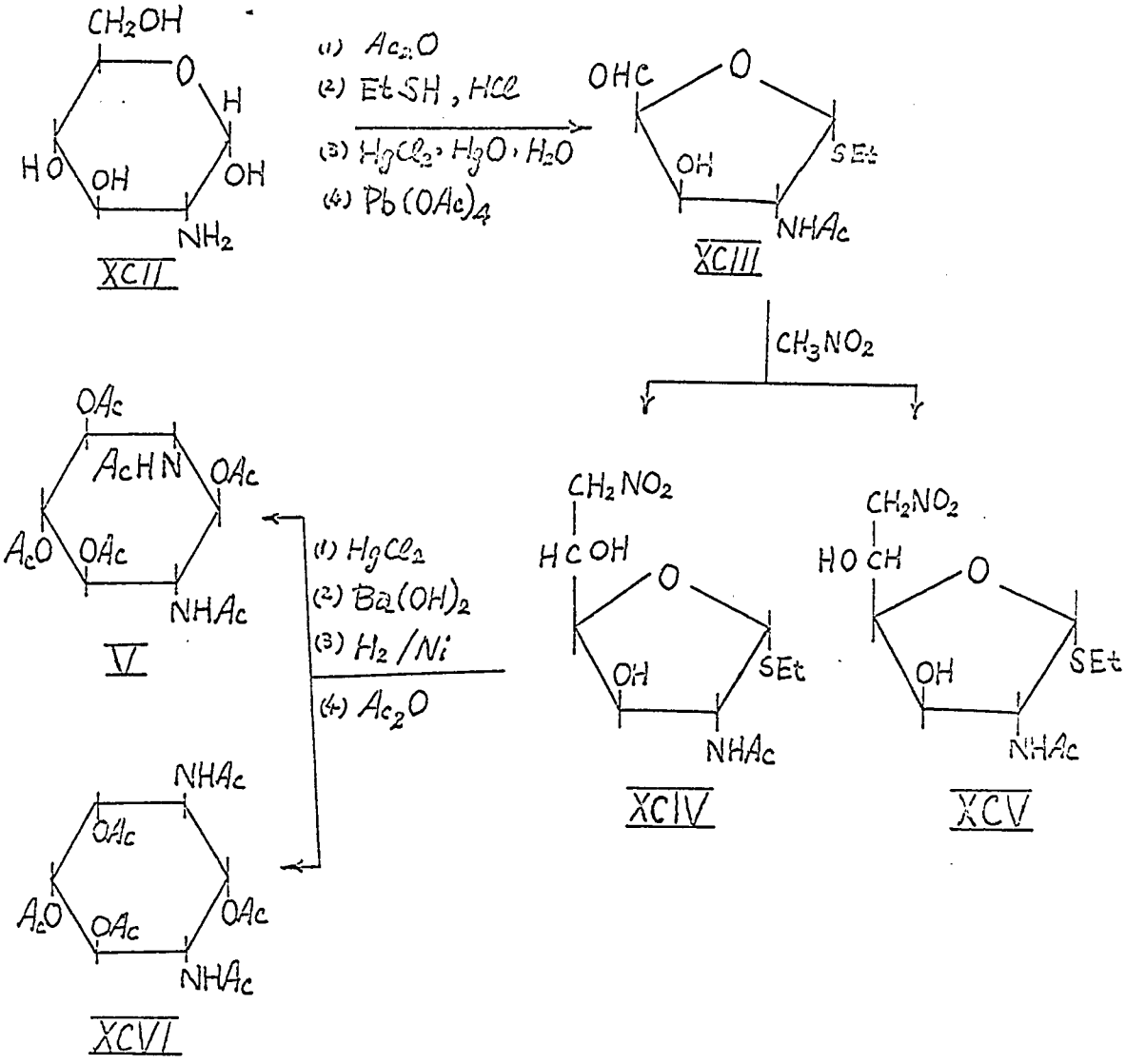
LXXXIX

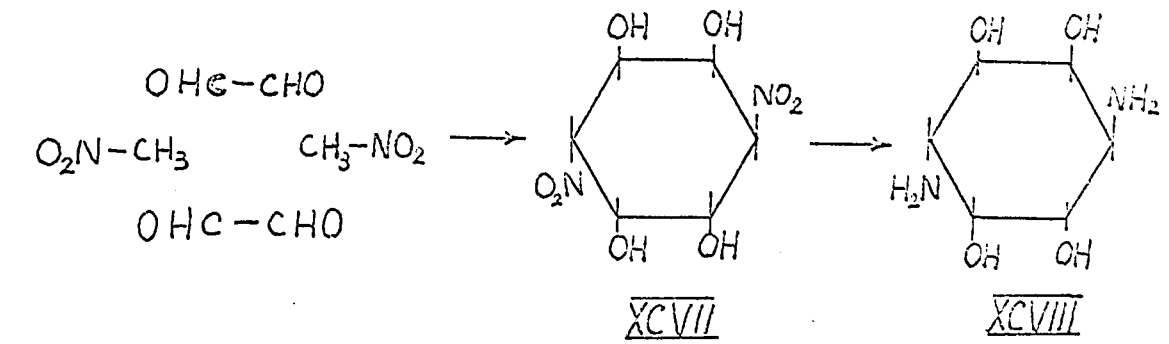
Wolfrom and associates (23) utilized the principle of Fischer's nitroinositol synthesis for the first synthesis of streptamine. D-Glucosamine (XCII) was converted, in a number of steps, into ethylthio 2-acetamido-2-deoxy-5-aldo-D-xylofuranoside (XCIII) which was condensed with nitromethane to give a mixture of ethylthio 2-acetamido-2,6-dideoxy-6-nitro-D-gluco- (XCIV) and L-idofuranosides (XCV). Hydrolysis of the thioglycosides with mercuric chloride was followed by alkalinization, whereby ring closure occurred and a mixture of 1,3-dideoxy-1-acetamido-3-nitro-inositols resulted. Reduction with Raney nickel and acetylation gave a readily separable mixture of hexaacetyl streptamine (V, major component) and of a stereoisomer of the probable formula XCVI (minor component).

Extending the nitromethane cyclization of dialdehydes (100,101), Lichtenthaler and Fischer investigated the reaction between nitromethane and glyoxal (102). They isolated a 1,4-dideoxy-1,4-dinitro-inositol (XCVII) that was reduced to the corresponding inosadiamine XCVIII and shown by n.m.r. studies to possess the neo configuration.

D. Reductions of Aromatic Compounds

As early as 1914, Wieland and Wishart (103) claimed to have obtained myo-inositol in more than 50% yield by catalytic hydrogenation of hexahydroxybenzene. The surprising stereoselectivity of this reaction prompted reinvestigations, especially since later workers were unable to confirm the


CH2OH
HO
OH
OH
NH2
XCII
(1) Ac2O
(2) EtSH, HCl
(3) HgCl2, HgO, H2O
(4) Pb(OAc)4
OHC
OH
SEt
NHAc
XCIII
CH3NO2
OAc
AcHN
OAc
AcO
OAc
NHAc
V
(1) HgCl2
(2) Ba(OH)2
(3) H2/Ni
(4) Ac2O
CH2NO2
HCOH
OH
SEt
NHAc
XCIV
CH2NO2
HOCH
OH
SEt
NHAc
XCV
NHAc
OAc
AcO
OAc
OAc
NHAc
XCVI


OHC-CHO
O2N-CH3
CH3-NO2
OHC-CHO
OH
OH
NO2
O2N
OH
OH
XCVII
OH
OH
NH2
H2N
OH
OH
XCVIII

results. Kuhn and coworkers (104) pointed out that the preparation of the palladium catalyst was of critical importance for the course of reaction. The stereochemical composition of the hydrogenation products was fully investigated by Angyal and McHugh (105) who found that considerable loss of oxygen by hydrogenolysis had occurred and the products ranged from cyclohexanetriols to inositols, the latter accounting for only 25-30% of the yield (Table II).

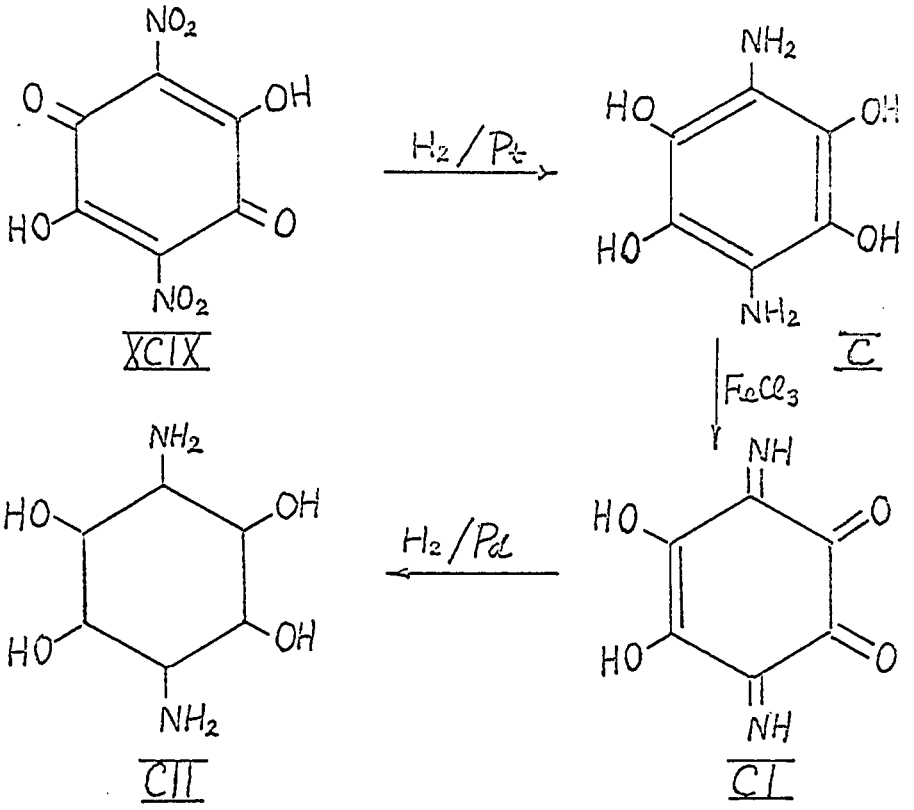
The possibility of obtaining aminocyclitols from aromatic compounds was first studied by Quadbeck and Röhm (106). Nitranilic acid (XCIX) was catalytically hydrogenated, in the presence of platinum, to give, in a yield of 70%, 1,4-diamino-tetrahydroxybenzene (C) (107). The latter compound was oxidized with ferric chloride to yield rhodizonic acid 1,4-diimine (CI) (108). The diimine CI was hydrogenated in the presence of palladium black to an inosadiamine-1,4 (CII) which was isolated as its sulfate. Similarly, triaminophloroglucinol (CIII) (109) was hydrogenated with platinum to give a 60% yield of an inosatriamine (CIV). No information on the configurations of inosadiamine CII and inosatriamine CIV has as yet been provided.

Table II

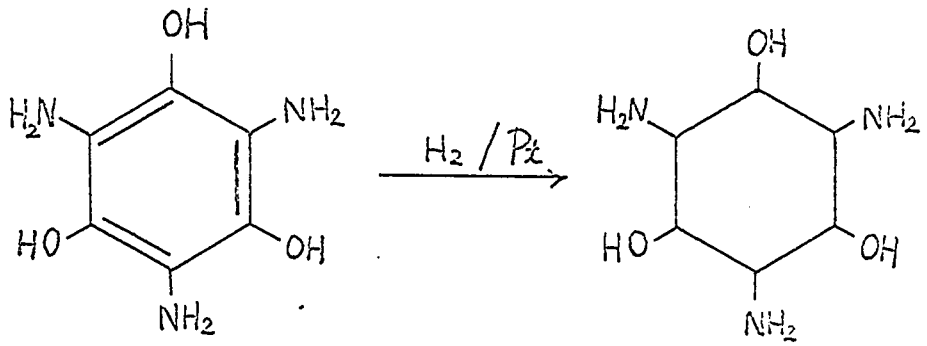
Products Isolated from the Hydrogenation of Hexahydroxybenzene

Compound	Yield (%) Pd, 20°, 1 atm	Ni, 120-140°, 150 atm	10% Pd-C 20°, 1 atm
Inositols:			
myo-(1235/46)	17.2	1.9	7.5
cis-(123456/)	1.7[a]	4.7	20.0
scyllo-(135/246)	1.2	0.1	1.0
epi-(12345/6)	0.2	1.8	0.3
(±)-(124/356)	2.9	---	0.05
neo-(123/456)	---	0.05	---
allo-(1234/56)	---	0.1	---
cis-Inosose	2.0	---	---
Quercitols:			
cis-(12345/)	2.7	8.1	0.4
epi-(1235/4)	0.5	1.9	---
scyllo-(135/24)	0.05	0.1	0.05
Cyclohexanetetrol (acetate, m.p.124°)	1.2	---	---
all-cis-Cyclohexane-1,2,3-triol	---	0.5	---
Reducing compound (m.p. 160-161°)	0.3	---	---

a A yield of 4.5% was obtained in a run in which PtO_2 was used to reduce any inososes remaining after the Pd-catalysed hydrogenation.



XCIX C CI CII



CIII CIV

SPECIFIC AIMS OF THIS THESIS

As has been shown in the Introduction, aminocyclitols are being discovered, in increasing numbers, in products of microbiological origin and of medicinal significance. Among those found to date there is, however, only one that has the structure of a 1,3-diamino-4,5,6-cyclohexanetriol, namely, 2-deoxystreptamine. In 1962, at the outset of this work, 2-deoxystreptamine had not yet been synthesized, nor had any one of its stereoisomers been described in the literature. The structure of 1,3-diamino-4,5,6-cyclohexanetriol allows the formation of 16 stereoisomers which include 4 meso forms and 6 pairs of enantiomorphs. Since it can be anticipated that some of these may be found in nature or may become desirable as starting materials for synthetic antibiotics, it was decided that attempts should be made to synthesize some members of this family and, if possible, 2-deoxystreptamine itself.

One feasible approach was seen in the catalytic hydrogenation of 4,6-dinitropyrogallol. Although this reaction would certainly not proceed in a stereospecific way, it seemed possible that some isomers would be formed preferentially and it was hoped that these could be separated and given configurational assignments.

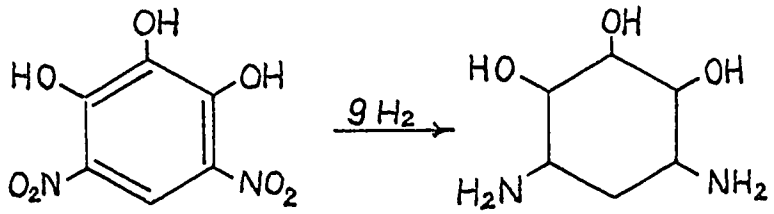
A stereospecific synthesis of 2-deoxystreptamine starting from D-xylose was to be attempted.

RESULTS AND DISCUSSION

Part I. Aminocyclitols from 4,6-Dinitropyrogallol

The problem to be solved can be divided into the following parts:

(1) Hydrogenation of 4,6-dinitropyrogallol (I)[a]



I II

(2) Separation of the reaction products.

It was expected that the reaction would yield several stereoisomers of 1,3-diamino-4,5,6-cyclohexanetriol (II), and the possibility of the formation of by-products could not be excluded.

(3) Characterization of the reaction products by gathering analytical and physical data and by preparing derivatives.

(4) Proof of structure of, and assignment of configuration to, the reaction products.

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a A new set of Roman numerals starts from hereon.

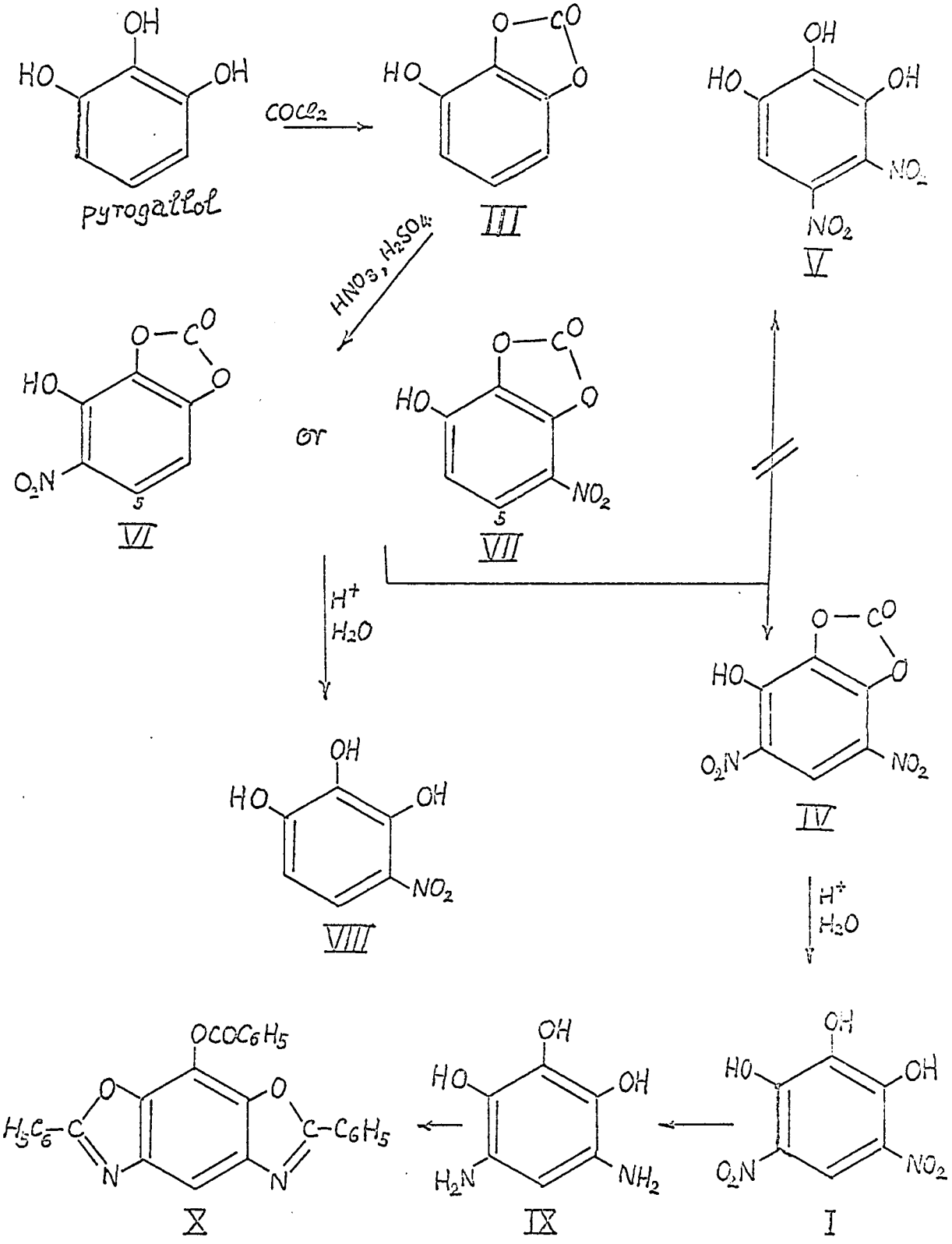
1. Catalytic Hydrogenation of 4,6-Dinitropyrogallol

A. 4,6-Dinitropyrogallol (I)

4,6-Dinitropyrogallol (I) was prepared according to Einhorn, Cobliner and Pfeiffer (110) by nitration of pyrogallol 1,2-carbonate (III), the latter being obtained from pyrogallol and phosgene. The nitration is carried out at low temperatures, and the dinitro carbonate (IV) is subsequently hydrolyzed without isolation, giving I in 79% yield.

We satisfied ourselves that the dinitropyrogallol obtained has structure I as proposed (110), and not the isomeric structure V. By arresting the nitration of III at an early stage it was found that the initial nitration product of III is a material (VI or VII) which, on hydrolysis, gives rise to 4-nitropyrogallol (VIII). In VI (or VII) the 5-position is deactivated by the nitro group (111, 112), so that the second nitro group should enter preferentially in meta-position to the first one. Moreover, the earlier authors (110) reduced I with tin and hydrochloric acid to the corresponding diamino compound (IX) which, with benzoyl chloride, afforded the bis-isoxazole X in proof of the substitution pattern in I.

In order to find out whether our preparation of I, though having the appearance of a sharp-melting uniform compound, might be contaminated with isomer V, n.m.r. spectra in three different solvents were taken. In all cases only a single, sharp proton signal was observed, a fact which supports the homogeneity of the preparation.


pyrogallol
III
V
VI
VII
or
VIII
IV
I
IX
X
$COCl_2$
HNO_3, H_2SO_4
H^+
H_2O

B. Catalytic Hydrogenations

Bearing in mind previous work on the catalytic hydrogenation of benzene derivatives to cyclitols (103-106, cf. Introduction, p. 41), 4,6-dinitropyrogallol (I) was hydrogenated in the presence of various catalysts and in different solvents, at room temperature and atmospheric pressure.

When I was hydrogenated in dilute hydrochloric acid in the presence of palladium on charcoal (10% Pd), the uptake of hydrogen was fast in the initial stage of reduction. After about six moles of hydrogen had been consumed as required to convert $2\ NO_2 \longrightarrow 2\ NH_2$, further hydrogenation during the next 20 hours became negligible despite addition of fresh catalyst. The filtrate from the catalyst became discolored (blue-violet) as soon as it was exposed to the air. Similar results were obtained with aqueous acetic acid as solvent and palladium black (104) as catalyst. The color could not be removed by means of activated charcoal, and removal of the solvents gave a blue-violet mass which was believed to be a mixture of diaminopyrogallol (IX) and products arising from its air oxidation. It was therefore concluded that palladium on charcoal and palladium black do not under ordinary conditions lend themselves to an exhaustive hydrogenation of I to the cyclitol stage, although Wieland and Wishart (103), Quadbeck and Röhm (106), and Angyal and McHugh (105) had been successful in analogous operations.

Hydrogenation of I in the presence of so-called "brown palladium oxyhydrate on barium sulfate" (113) has also been attempted. When I was suspended in dilute sulfuric or hydrochloric acid, the reduction of the two nitro groups was very fast with this catalyst. Further uptake of hydrogen was very slow despite the addition of more catalyst, but a total of nine moles was eventually reached. Work-up gave an impure crystalline material amounting to 64-68% of the starting material. Infrared spectroscopy revealed that it consisted mainly of IX (in salt form) along with several products, presumably aminocyclitols, in small quantities as shown by paper chromatography. An attempt to separate these crude products by column chromatography on Dowex 1-X2 (borate form) (114) was not successful. It was concluded that brown palladium oxyhydrate on barium sulfate was not satisfactory for the present purpose although it appeared to be somewhat more suitable than other palladium catalysts.

The hydrogenation of I was finally performed in a more satisfactory manner using Adam's catalyst (prehydrogenated platinum dioxide). The reactions were carried out in 50% aqueous acetic acid, 1/5 N sulfuric acid, and 1/3 N hydrochloric acid, at room temperature and atmospheric pressure. With the former two solvents the patterns of reaction products as shown by paper chromatography were quite similar, whereas with hydrochloric acid the pattern was substantially different and, for reasons explained below, less desirable. In all three sets of conditions the hydrogen uptake was rapid during the first stage (reduction

of the nitro group) and comparatively slow but steady enough during the second stage (hydrogenation of the benzene ring). Total reaction times were 13-15 hours in mineral acids and 50 hours in acetic acid. Survival of aromatic compounds as evidenced by discoloration upon exposure to air was slight; whatever remained of aminophenol-like products could be removed by air oxidation followed by treatment with activated charcoal. The aliphatic amines formed were then isolated as crystalline mixtures of hydrochlorides or sulfates in yields of 40%, 29%, and 46% (hydrogenation in acetic acid, sulfuric acid, and hydrochloric acid, respectively). Paper chromatography on borax paper of the crude reaction products showed that each consisted of a large number of components in the R_{ds} [a] range 0.74 to 2.15. It was later established that the products of R_{ds} 1.15 (and greater) had lost one or more functional groups presumably due to hydrogenolysis. These faster-moving products were especially prominent in the hydrogenation in hydrochloric acid; it is for this reason that this solvent must be regarded as the least suitable one (Fig. 5).

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a) R_{ds}, chromatographic speed relative to authentic 2-deoxystreptamine, a sample of which was kindly provided by Dr. I.R.Hooper of Bristol Laboratories, Inc., Syracuse, N.Y.

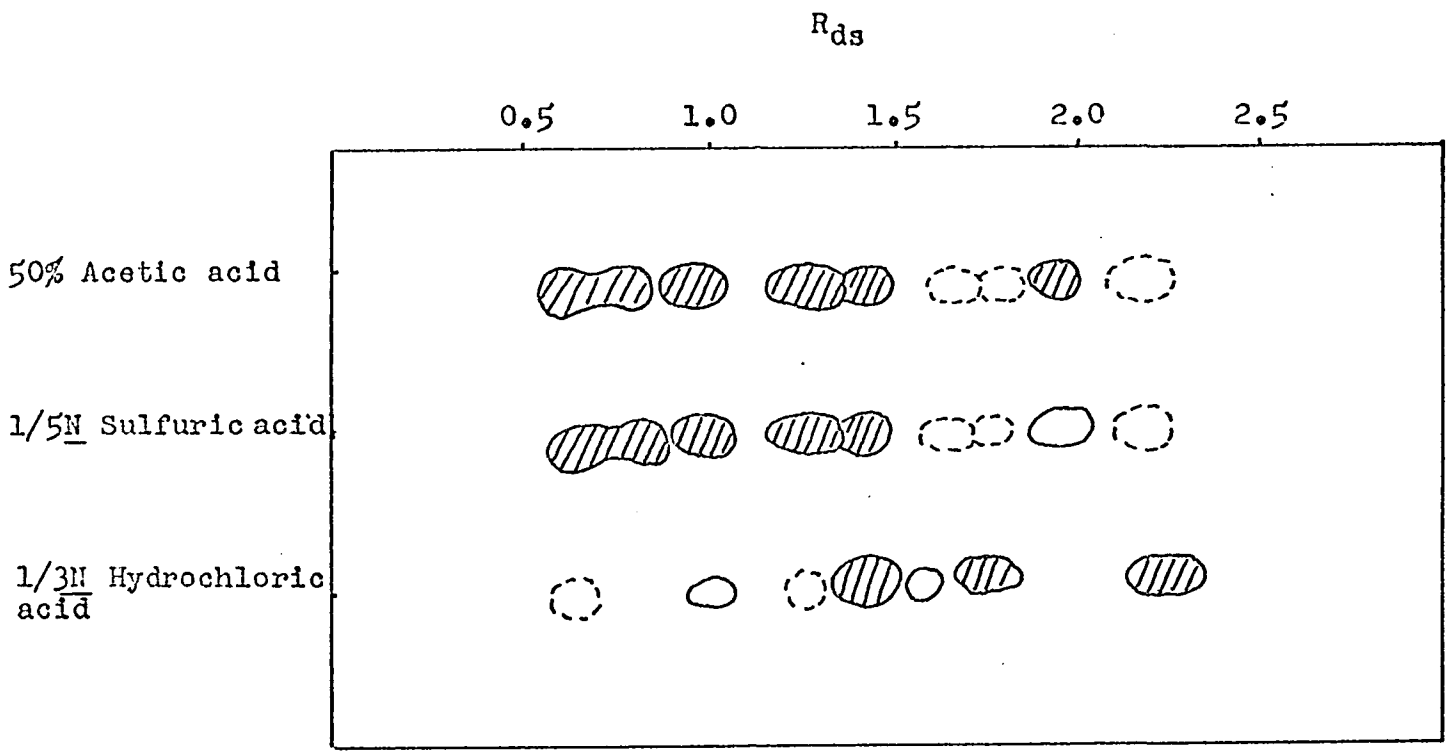


Fig. 5. Paper chromatograms on borax paper of the crude products obtained from the catalytic hydrogenation of 4,6-dinitro-pyrogallol in three different solvents. Intensities; ◌ weak, ○ medium, ▨ strong.

2. Separation of the Hydrogenation Products

Preliminary attempts to separate the complex mixtures of hydrogenation products by fractional crystallization were unsuccessful. It was then tried to achieve separation by chromatography using a column of powdered cellulose. With this method and with acetone-water (4:1, v/v) as irrigating system, Angyal and McHugh (105) have successfully separated mixtures of inositols. However, our products failed to separate, and when a different solvent system (pyridine-ethyl acetate-water-acetic acid (5:5:3:1, v/v) (115)) was employed, a partial but unsatisfactory separation was observed.

Better results were finally achieved by chromatography on ion exchange columns. A procedure was elaborated that comprised an application of the gradient elution technique on cation exchange resin, Dowex 50 (H^+), as well as the use of anion exchange columns (Dowex 1-x2) in the borate and hydroxyl forms. The procedures employed were modifications of those used for the separation of carbohydrates by Horowitz, Roseman and Blumenthal (116), Khym and Zill (114), and Austin, Hardy, Buchanan and Baddiley (117). It was thus possible to obtain aminocyclitols in pure, crystalline and chromatographically homogeneous condition; in addition, ammonium chloride was isolated which had obviously arisen from a partial deamination occurring during the hydrogenation. The total recovery of isolated entities from the columns was 32.5% of the mixture put on. The remainder was contained in

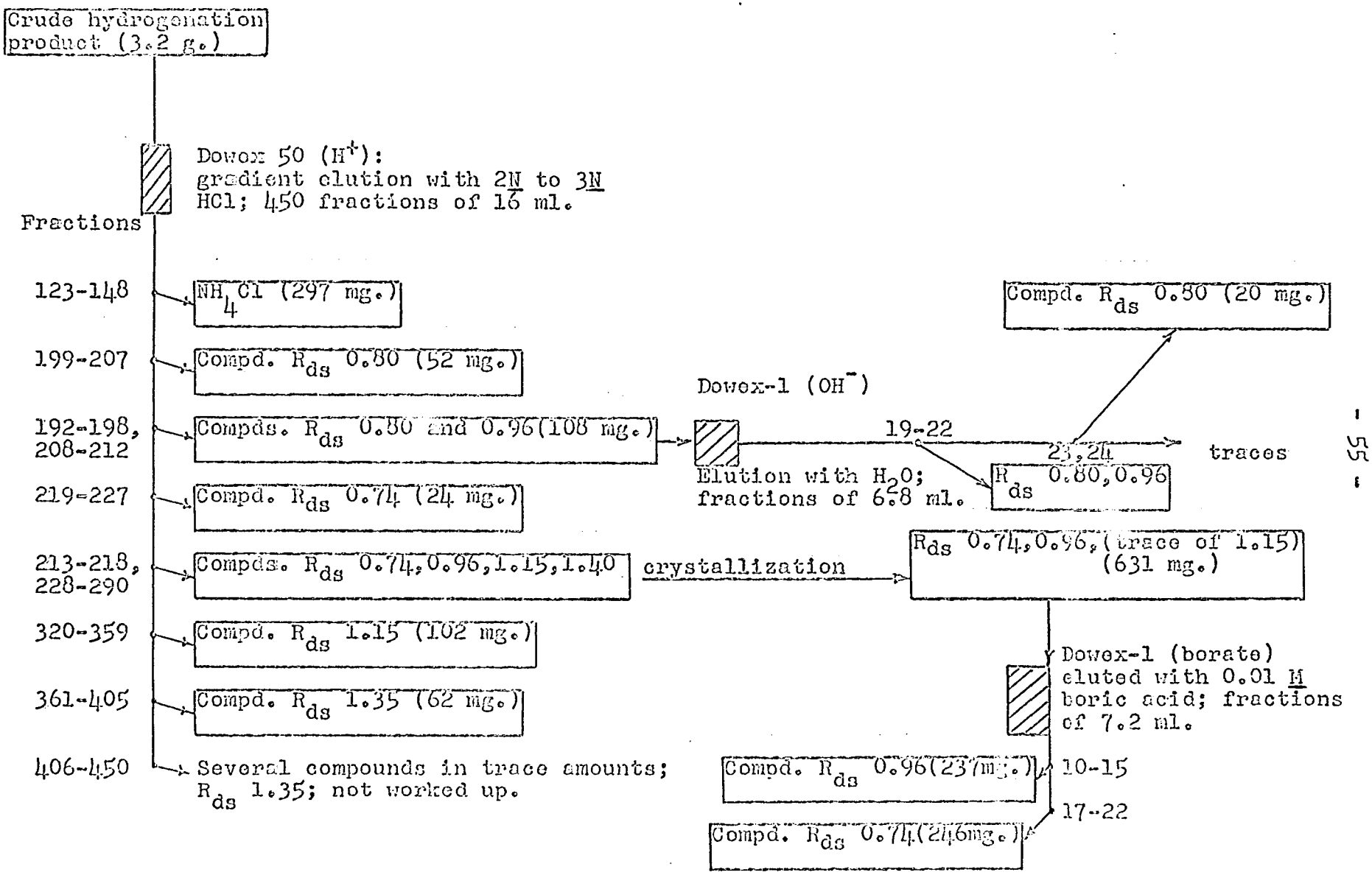


Fig. 6 Separations of crude hydrogenation product by ion-exchange resins.

interfractions or could not be eluted under the conditions employed. The course of a typical fractionation is represented by the flow-sheet in Fig. 6. It is seen from the figure that the following compounds were isolated (in parentheses, the yields based on the crude mixture are given):

Compound R_{ds} 0.74	(8.4%)
Compound R_{ds} 0.80	(2.25%)
Compound R_{ds} 0.96	(7.4%)
Compound R_{ds} 1.15	(3.2%)
Compound R_{ds} 1.35	(1.95%)
Ammonium Chloride	(9.3%)
	32.50%

The mother liquors left after isolation of five amino-cyclitols and ammonium chloride showed, as indicated by paper chromatography, that they still contained quite a few other amino-cyclitols. No further attempt was made to separate them since they did not appear to be present in sufficient amounts for isolation, and moreover, most of them had suffered loss of functional groups.

3. Characterization of the Products

The five hydrogenation products whose isolation has been described in the preceding section were obtained as colorless, crystalline salts which gave, in agreement with their expected structures as aminoalcohol hydrochlorides, blue to violet color reactions with ninhydrin and positive tests for chloride ion. Their infrared spectra were also in accord with this, as they showed strong hydroxyl absorption in the regions 3300-3400 and 1010-1050 cm^{-1}, and bands due to $-NH_3^+$ in the regions of 1575-1590 and 1480-1490 cm^{-1}. Upon heating, the compounds decomposed over a wide range from 250-300° without distinct melting, a feature common to hydrochlorides of polyhydroxy-amino derivatives, particularly in the carbohydrate series. In addition to their different chromatographic mobilities the products are readily distinguished by their x-ray powder diagrams (Fig. 7).

Elemental analyses confirmed that the three slow-moving products (R_{ds} 0.74, 0.80, 0.96) had the composition of diamino-trihydroxycyclohexane dihydrochlorides, whereas the two faster-moving products (R_{ds} 1.15 and 1.35) were revealed to possess the composition of diamino-dihydroxycyclohexane dihydrochlorides. It must be concluded that these latter compounds had arisen from I through hydrogenolytic loss of a hydroxyl group. Similar cleavages have been observed elsewhere before (105).

In order to characterize the compounds further, and to obtain substances which would lend themselves to structural

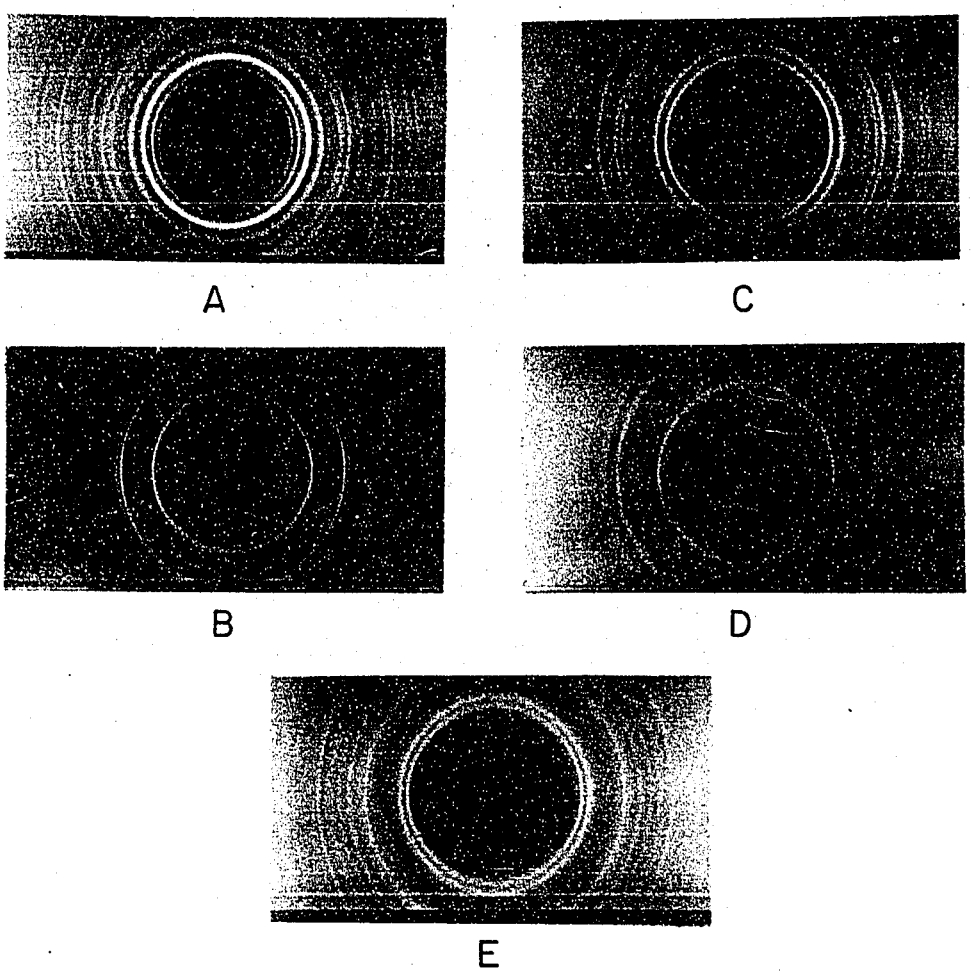


Fig. 7. Powder x-ray diffraction diagrams of the dihydrochlorides of

A, compound R_{ds} 0.74
B, compound R_{ds} 0.80
C, compound R_{ds} 0.96
D, compound R_{ds} 1.15
E, compound R_{ds} 1.35

and configurational studies by periodate oxidation and n.m.r. spectroscopy, the following derivatives were made.

Compound R_{ds} 0.74 was converted into a crystalline dipicrate and a crystalline pentaacetate, both of which gave correct elemental analyses. The pentaacetate (m.p. 285-287°, dec.) showed an abnormality which is, however, not without precedent. The compound appeared to be of considerable polarity, in that it could not be extracted with chloroform from aqueous solution. Similar instances have been reported in the literature. The crystalline hexaacetate of streptamine that was prepared by Wolfrom (23) was water-soluble and insoluble in chloroform, and Folkers (17) obtained streptamine hexaacetate in a water-soluble and in a chloroform-soluble form. No explanations have been given for these observations.

Compound R_{ds} 0.80 yielded a semi-solid pentaacetate. It was soluble in chloroform.

Compound R_{ds} 0.96 furnished a crystalline N,N'-diacetate by selective N-acetylation with acetic anhydride in water in the presence of Dowex 1-X2 ($CO_3^{=}$) anion exchange resin (118). Peracetylation gave a syrupy pentaacetate.

Compound R_{ds} 1.15 similarly afforded a crystalline N,N'-diacetate. By peracetylation, two crystalline products were obtained, both of which gave elemental analyses corresponding to diacetamido-diacetoxycyclohexane. They differed in their solubilities: one of them, obtained in small yield, exhibited the characteristics that one would normally expect of a fully acetylated

product. It was extracted with chloroform from the acetylation mixture and was recrystallized from chloroform-ether. The major product, obtained in 86% yield, had a surprisingly high polar character; it was not extractable from aqueous solution by chloroform, and it crystallized from ethanol upon addition of chloroform.

Compound R_{ds} 1.35 gave a chloroform-soluble tetraacetate in syrupy condition.

The infrared spectra of all the fully acetylated compounds just described showed the expected ester and amide bands and lacked hydroxyl absorption. The details are given in the Experimental Part.

None of the infrared spectra of the hydrochlorides isolated, nor of the acetyl derivatives prepared, was identical with spectra obtained from deoxystreptamine dihydrochloride and its acetyl derivatives. It is thereby established that the hydrogenation of I has afforded new products, as indeed the R_{ds}-values have already revealed.

4. Structures and Configurations

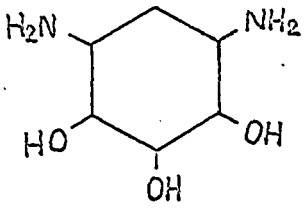
A. Periodate Oxidation Studies

Periodate oxidation is used extensively as a diagnostic means of ascertaining the presence of glycol groupings. Its manifold applications in carbohydrate chemistry have been reviewed (119). If the only oxidizable grouping in a compound comprises two vicinal hydroxyls, one mole of periodate will be consumed; if the grouping comprises three contiguous hydroxyls, two moles will be consumed. Free amino groups react like hydroxyl groups, whereas N-acylated amino groups do not.

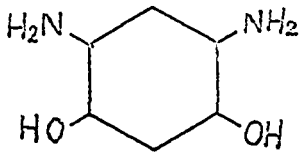
The three aminocyclitols of R_{ds} 0.74, 0.80 and 0.96, which in the preceding section have been described as giving analyses corresponding to diamino-trihydroxycyclohexane, after N-acetylation consumed close to two moles of periodate. This confirmed that the structures had retained the 1,2,3-triol system of the starting dinitropyrogallol (I) and permitted their classification as stereoisomers of 2-deoxystreptamine (XI).

The two aminocyclitols of R_{ds} 1.15 and 1.35, described in the preceding section as having the composition of diamino-dihydroxycyclohexane, consumed after N-acetylation, only 0.10 and 0.19 mole of periodate, respectively. This consumption was far below the one mole that would have been expected for vicinal diols, and the two aminocyclitols were therefore assigned the structure of 1,3-diamino-4,6-dihydroxycyclohexane. The fact that there did occur some oxidation can probably be

attributed to incomplete N-acetylation under the conditions of the assay.



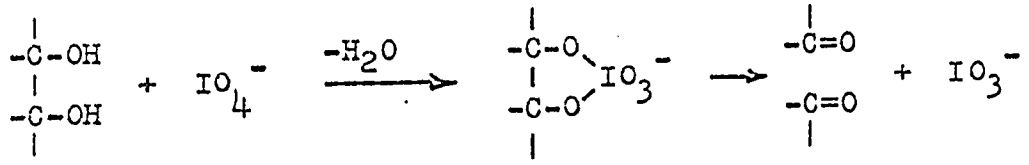
Structure of compounds
R_{ds} 0.74, 0.80, and 0.96



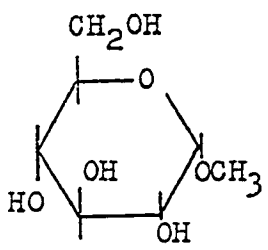
Structure of compounds
R_{ds} 1.15 and 1.35

Relative rates of periodate oxidation in 5- and 6-membered cyclic glycols have frequently been measured in order to distinguish between cis and trans configurations (120). Thus, cis-1,2-cyclohexanediol is oxidized about 30 times more rapidly than the trans-isomer (121). Similarly, in 1,2,3-cyclohexanetriols, the cis-cis isomer is oxidized more rapidly, and the trans-trans isomer more slowly, than the cis-trans isomer (122). Methyl α-D-glucopyranoside (XII), with a trans-trans triol arrangement, reacts more slowly than methyl α-D-mannopyranoside (XIII) which contains a cis-trans triol grouping (123). Of the various inositols the all-cis isomer is oxidized fastest and the all-trans isomer, most slowly (124). This general experience has been employed in the assignment of configurations to some inosamines (125) and in the elucidation of 2-deoxystreptamine (55). According to Criegee (126), the differential oxidation rates in cis- and trans-glycols are explained by the formation of cyclic esters of periodic

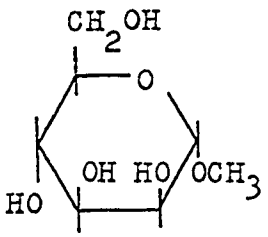
acid in the rate-controlling step, where cis-glycols form the 5-membered ring intermediate more easily than trans-glycols (119):



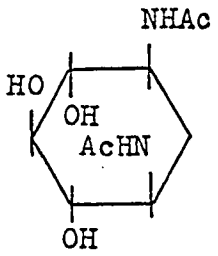
We have compared the rates of periodate consumption at 3° of the three new diaminocyclohexanetriols after N,N'-diacetylation, with the rates for methyl α-D-glucopyranoside (XII), methyl α-D-mannopyranoside (XIII), and N,N'-diacetyl-2-deoxystreptamine (XIV). The results are depicted in Fig. 8 and are evaluated as follows.



XII



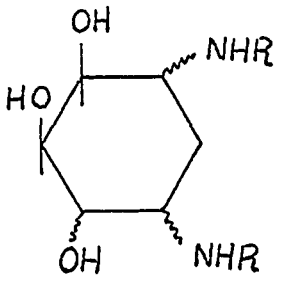
XIII



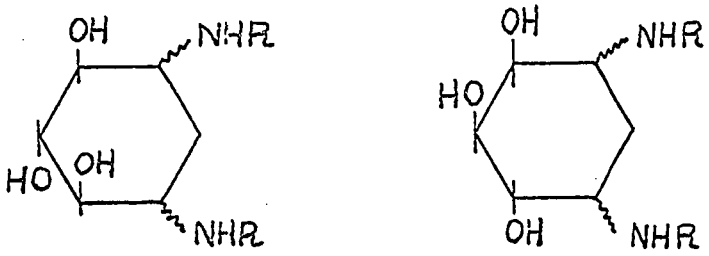
XIV

Compound R_{ds} 0.74 (Fig. 8 (b)).- The consumption of the first mole of periodate was faster than in the mannoside XIII, and much faster than in the trans-trans triols, glucoside XII and

2- deoxystreptamine XIV. This suggests the presence of at least one cis-glycol grouping:



Compound R_{ds} 0.80 (Fig. 8 (c)).- The periodate consumption appeared to be faster than in the trans-trans triols XII and XIV, but not significantly so. It was slower than in the cis-trans compound XIII. No definite conclusion could be reached except that a cis-cis arrangement was ruled out. Both cis-trans and trans-trans arrangements appeared possible:



Possible configurations

Compound R_{ds} 0.96 (Fig. 8 (d)).- The extremely fast consumption of two moles of periodate suggested the presence of a

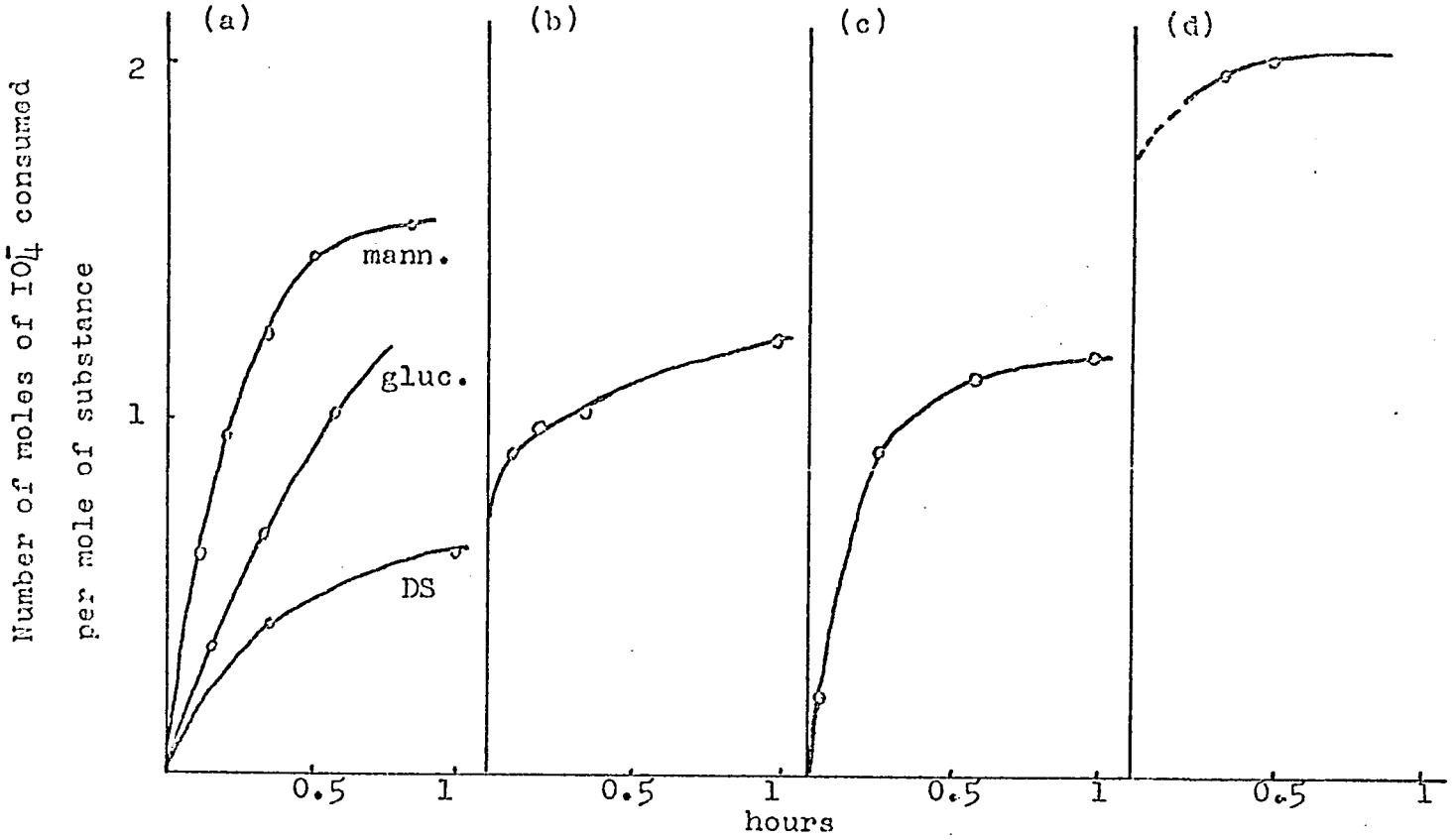
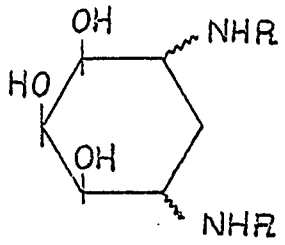


Fig. 8. Rate of periodate oxidations: (a) methyl-α-D-mannoside(mann.), methyl-α-D-glucoside(gluc.), N,N'-diacetyl-2-deoxystreptamine(DS); (b) N,N'-diacetate of compound R_{ds} 0.74; (c) N,N'-diacetate of compound R_{ds} 0.80; (d) N,N'-diacetate of compound R_{ds} 0.96.

cis-cis-triol grouping:



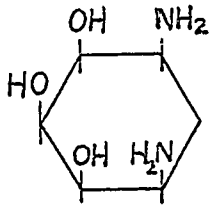
Most likely configuration

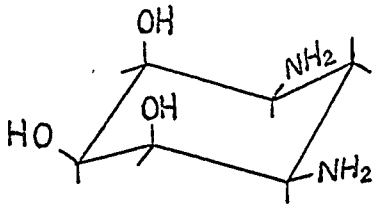
B. Nuclear Magnetic Resonance Studies

Nuclear magnetic resonance spectroscopy has finally been employed to complete the partial formulas that had emerged from the periodate data. On the basis of arguments which will be presented subsequently, the following assignments were made:[a]

Compound R_{ds} 0.74 :

1α, 3α-diamino-4α,5α,6α-trihydroxycyclohexane (XV)





XV

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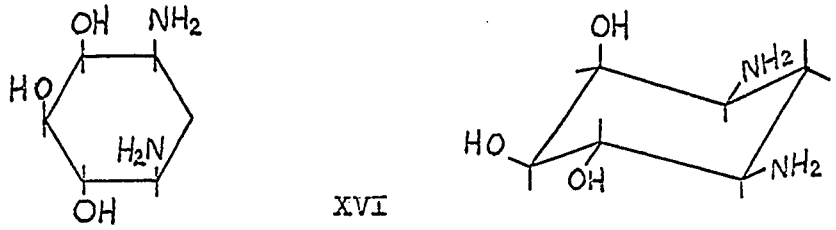
a Substituents on that side of the ring which has the most substituents are designated "α"; substituents on the opposite side are designated "β".

Compound R_{ds} 0.80 :

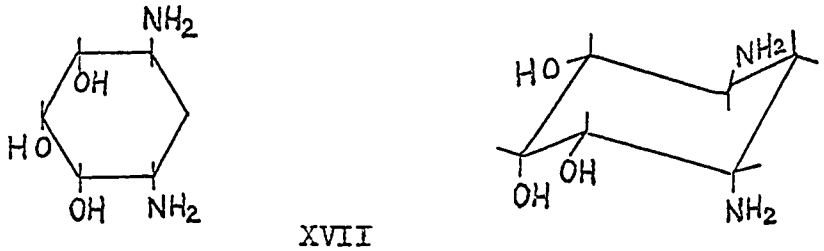
D,L-1α,3α-diamino-4α,5α,6β-trihydroxycyclohexane (XVI)



XVI

Compound R_{ds} 0.96 :

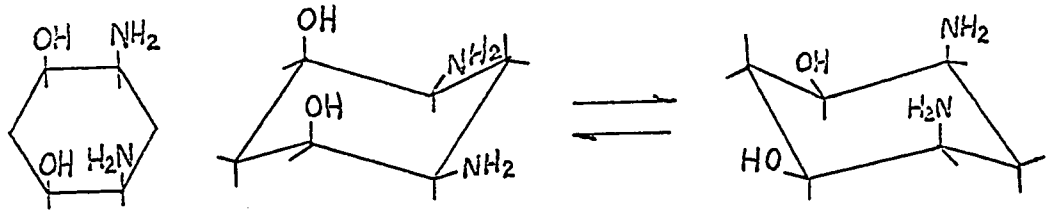
D,L-1α,3β-diamino-4α,5α,6α-trihydroxycyclohexane (XVII)



XVII

Compound R_{ds} 1.15 :

1α,3α-diamino-4α,6α-dihydroxycyclohexane (XVIII)



XVIII

Compound R_{ds} 1.35 :

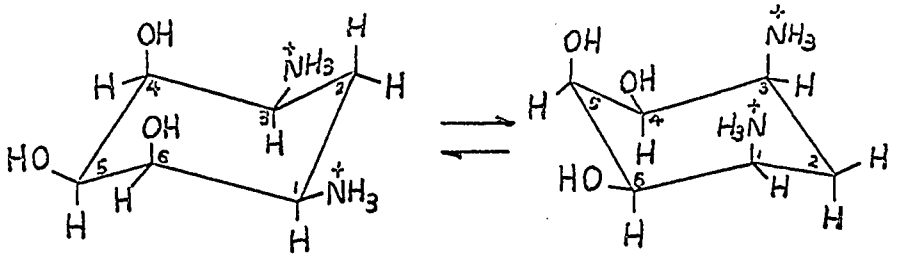
D,L-1α,3α-diamino-4α,6β-dihydroxycyclohexane (XIX)

XIX

(1) 1α,3α-Diamino-4α,5α,6α-trihydroxycyclohexane (XV)

The n.m.r. spectrum of compound R_{ds} 0.74 (dihydrochloride) in deuterium oxide is shown in Fig. 9. The signals for the hydrogens of the methylene group appeared in the region of 7.7τ. Unfortunately, they were bunched together so that they could not be used to establish the orientation of the protons at C-1 and C-3 in the way Lemieux (58) had used the analogous signals of 2-deoxystreptamine. However, a clearly resolved triplet of intensity two at 5.67τ, next to the DOH signal, could be assigned to the two protons at C-4 and C-6. This assignment was based on the fact that the two positively-charged nitrogen ions vicinal to them exert a strong deshielding effect, shifting the signal to low field. Such an effect was observed by Lemieux (58) in 2-deoxystreptamine and was also reported by Slomp and Macfellar (62) for the spectrum of N,N'-dimethyl-actinamine dihydrochloride. The spacing of 2.6 c.p.s. of the triplet requires each of these two hydrogens to be either equatorial and coupled with two neighboring axial hydrogens, or axial and coupled with two neighboring equatorial hydrogens at C-5, and at C-3 and C-1. In the former case, the two amino groups at C-1 and C-3, and the hydroxyl group at C-5 would be equatorially oriented, and the resulting configuration would be XV. In the latter case, the two amino groups and the hydroxyl group at C-5 must be axially oriented and the resulting formula would be XX. As can be seen, XV and XX are two conformational

forms of one and the same configuration. Because of greater steric and polar substituent interaction, XX must be considered to be less stable.



XV XX

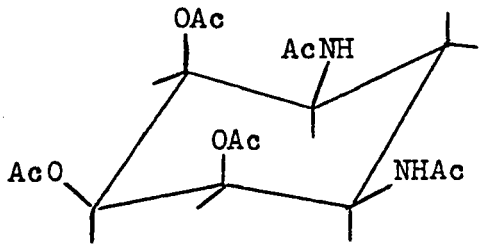
Additional proof for the configuration shown (XV) came from the n.m.r. spectrum of the pentaacetate (XXI) (Fig.10). Three prominent signals of intensities corresponding to six, three and six protons were observed at 7.73, 7.94 and 8.00 τ, respectively. The signal at 7.73 τ was assigned to two <u>axial</u> acetoxyl groups (at C-4 and C-6), the signal at 7.94τ to an <u>equatorial</u> acetoxyl group (at C-5), and the signal at 8.00τ to two <u>equatorial</u> acetamido groups. These assignments were based on the rule, first propounded by Lemieux <u>et al.</u> (127) and often since applied (128), that in the cyclohexane series <u>axial</u> acetoxyl signals appear at lower field than <u>equatorial</u> acetoxyl signals. A similar relationship exists between acetamido signals, and ample data are available to the effect that <u>equatorial</u> acetamido protons resonate at higher field than <u>equat.</u> acetoxyl protons (22, 128, 129). In the literature quoted, the following ranges

have been set forth:

ax.	$OCOCH_3$	7.78 - 7.87 τ
eq.	$OCOCH_3$	7.94 - 8.02 τ
ax.	$NHCOCH_3$	7.92 - 7.94 τ
eq.	$NHCOCH_3$	8.05 - 8.09 τ

These values pertain to chloroform solutions. In deuterium oxide we find slightly lower values throughout.

In conclusion, the spectra of the dihydrochloride and of the pentaacetate, together with the periodate data (p.65) which had required at least one (but allowed two) cis glycol groupings, establish compound F_{ds} 0.74 to be 1α,3α-diamino-4α,5α,6α-trihydroxycyclohexane (XV).



XXI

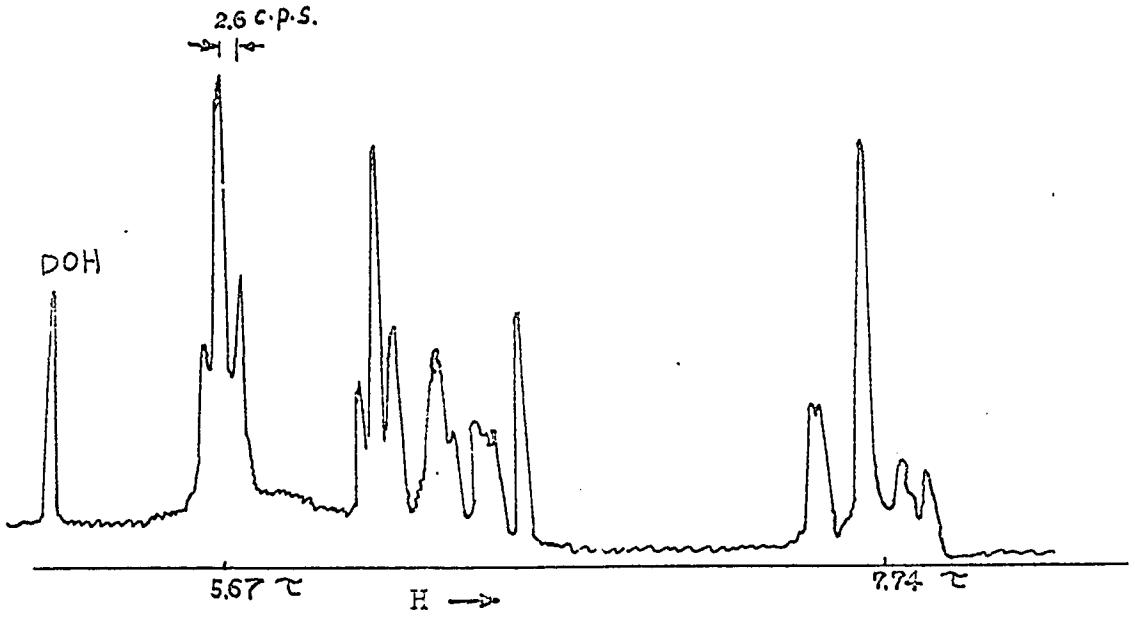


Fig. 9. N.M.R. Spectrum of 1α,3α-diamino-4α,5α,6α-trihydroxycyclohexane (XV) dihydrochloride in deuterium oxide.

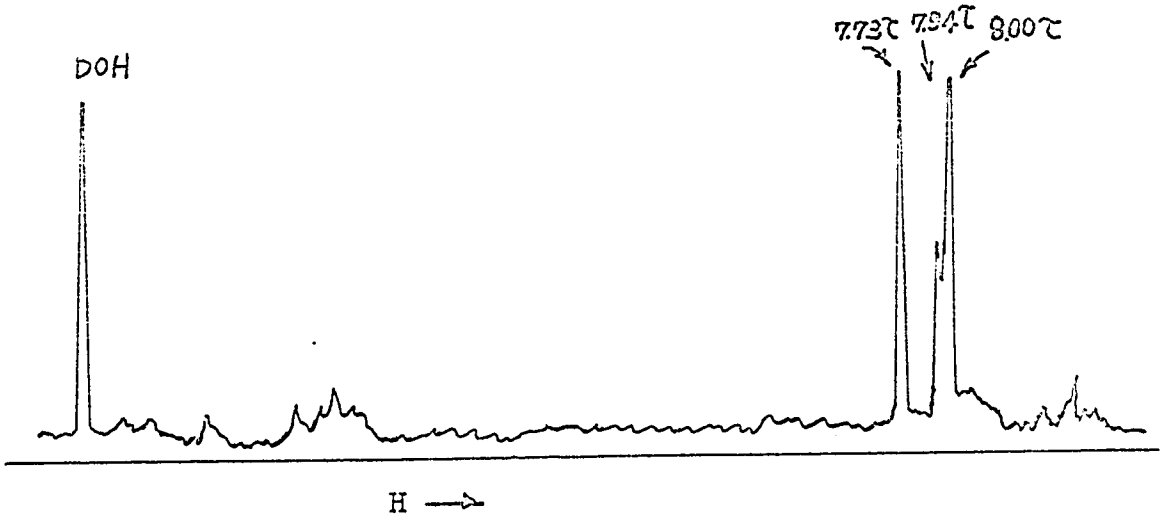
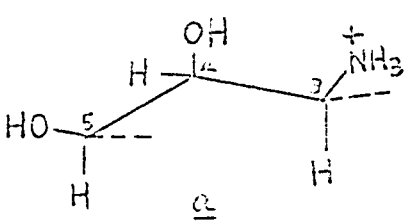


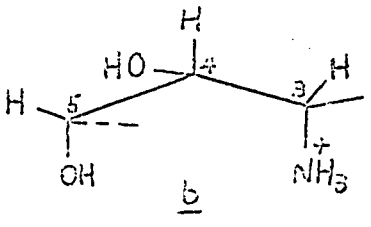
Fig. 10. N.M.R. Spectrum of 1α,3α-diacetamido-4α, 5α,6α-triacetoxycyclohexane (XXI) in deuterium oxide.

(2) D,L-1α,3α-Diamino-4α,5α,6β-trihydroxycyclohexane (XVI)

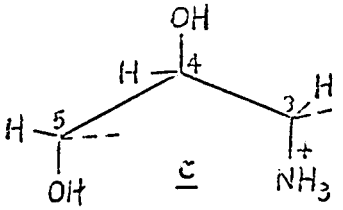
The n.m.r. spectrum of the aminocyclitol R_{ds} 0.80 (dihydrochloride) in deuterium oxide is shown in Fig. 11. The signals were bunched together except for a clearly resolved triplet of intensity one at lowest field (5.76 τ). The triplet was assumed to be due to a hydrogen (at C-4 or C-6) situated vicinal to one of the amino groups (cf. the deshielding effect mentioned under (1), above). Signals of the hydrogen vicinal to the other amino group appeared to be grouped together with other signals at higher field. The difference in chemical shift of these two structurally equivalent hydrogens at C-4 and C-6 showed that the molecule is not symmetrical. The spacing of the triplet was 2.5 c.p.s. which required the proton to be (i) equatorial and vicinal to two axial protons (partial formula a) (ii) axial and vicinal to two equatorial protons (partial formula b) (iii) equatorial and vicinal to two equatorial protons (partial formula c) or (iv) equatorial and vicinal to one equatorial and one axial protons (partial formulas d or e).



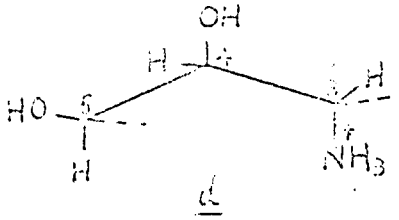
a



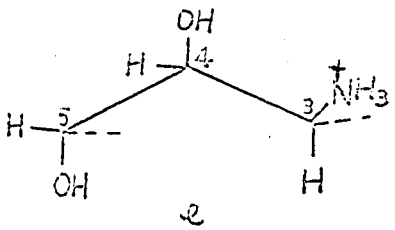
b



c



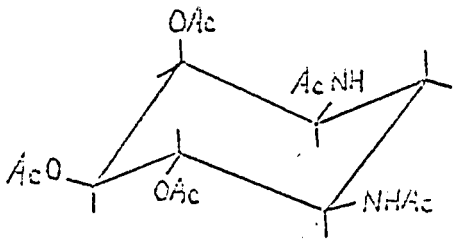
d



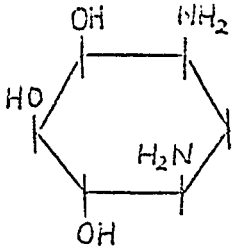
e

Each of the above five partial formulas can theoretically give rise to four complete formulas. However, only three of them are possible in the present case, since the molecule is not symmetrical as mentioned above. Thus, one could write three complete formulas, $\underline{a}_1$, $\underline{a}_2$ and $\underline{a}_3$ from the partial formula $\underline{a}$; and in the same way $\underline{b}_1$, $\underline{b}_2$ and $\underline{b}_3$ from $\underline{b}$; $\underline{c}_1$, $\underline{c}_2$ and $\underline{c}_3$ from $\underline{c}$; $\underline{d}_1$, $\underline{d}_2$ and $\underline{d}_3$ from $\underline{d}$; and $\underline{e}_1$, $\underline{e}_2$ and $\underline{e}_3$ from $\underline{e}$. Among these fifteen formulas only six ($\underline{a}_1$, $\underline{a}_3$, $\underline{b}_1$, $\underline{c}_2$, $\underline{d}_1$ and $\underline{e}_1$) are independent, since $\underline{a}_2$ and $\underline{b}_1$, $\underline{b}_2$ and $\underline{a}_1$, $\underline{b}_3$ and $\underline{a}_3$, $\underline{e}_2$ and $\underline{c}_2$ are enantiomorphs, and $\underline{c}_1$ and $\underline{d}_1$, $\underline{c}_3$ and $\underline{a}_1$, $\underline{d}_2$ and $\underline{b}_1$, $\underline{d}_3$ and $\underline{e}_1$, $\underline{e}_3$ and $\underline{a}_3$ have the same formulas(page 73a).

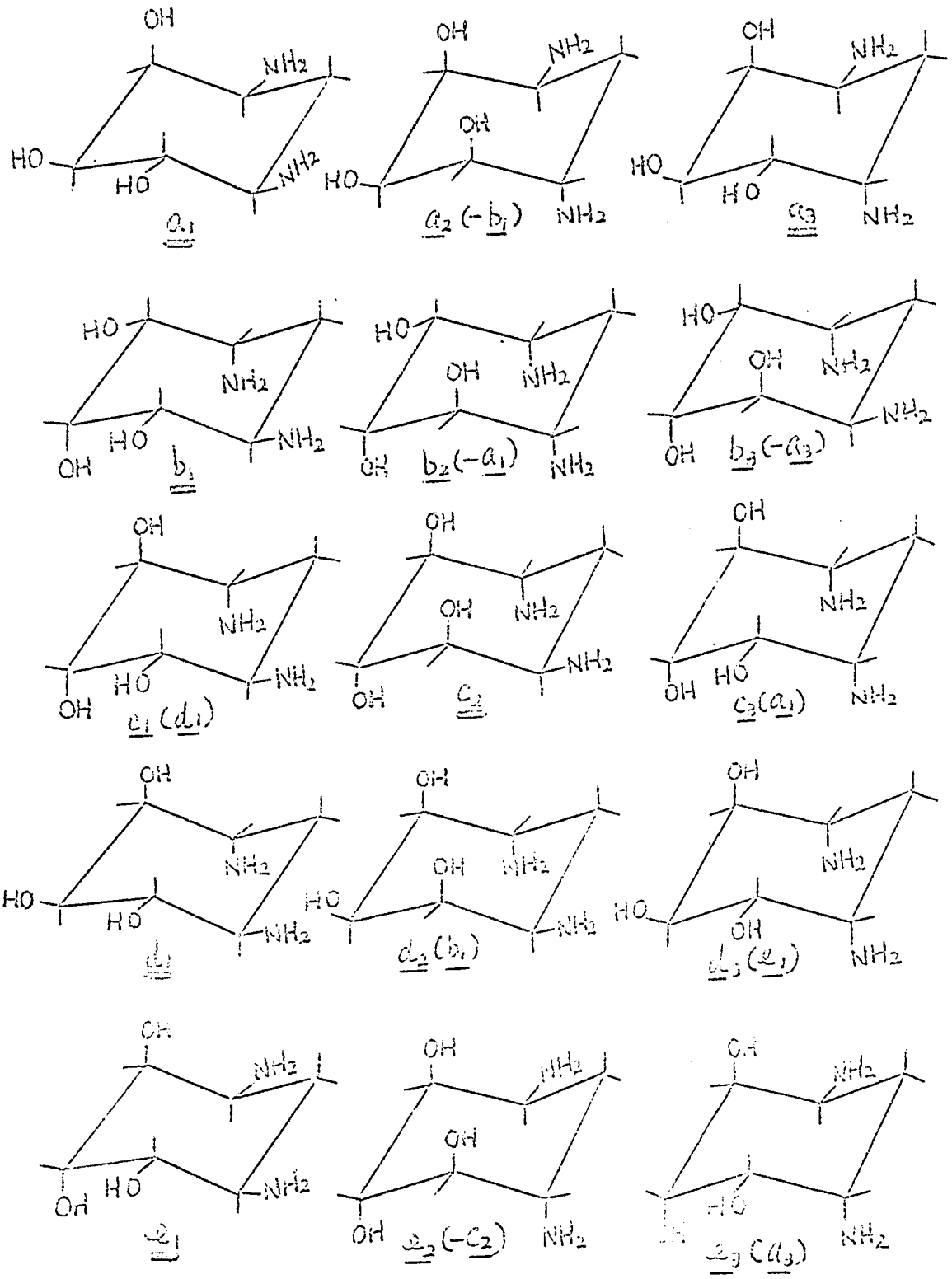
The spectrum of the pentaacetate in deuterochloroform (Fig. 12) showed five peaks of about equal intensities, each corresponding to 3 protons, at 7.83, 7.93, 7.95, 8.04, and 8.07 τ. According to the rules mentioned under (1), above, these signals were assigned to one axial acetoxyl group, two equatorial acetoxy groups, and two equatorial acetamido groups. It appears that among the six possible formulas ($\underline{a}_1$, $\underline{a}_3$, $\underline{b}_1$, $\underline{c}_2$, $\underline{d}_1$ and $\underline{e}_1$) mentioned above, only the formula $\underline{a}_1$ fits the above requirements, and the complete formula XXII for the pentaacetate could thus be written.



XXII



XVI


a1
a2 (- b1)
a3
b1
b2 (- a1)
b3 (- a3)
c1 (d1)
c2
c3 (a1)
d1
d2 (b1)
d3 (c1)
e1
e2 (- c2)
e3 (a3)

The formula of $\underline{c}_2$ or $\underline{e}_1$ cannot accommodate the data obtained from the pentaacetate spectrum and was therefore dismissed. The formula of $\underline{b}_1$, which shows a cis-cis relationship of the three hydroxyl groups, is excluded based on the periodate data (p. 65). However, the possibility of the formula $\underline{a}_3$ or $\underline{d}_1$ cannot be absolutely excluded if one assigns the signal of 8.04 τ to one equatorial acetoxyl group instead of one equatorial acetamido group notwithstanding a slight higher chemical shift found. The molecule represented by formula XVI is asymmetrical; since the product obtained in the synthesis arose from a symmetrical starting material it is presumably a racemate : D,L-1α,3α-diamino-4α,5α,6β-trihydroxy-cyclohexane.

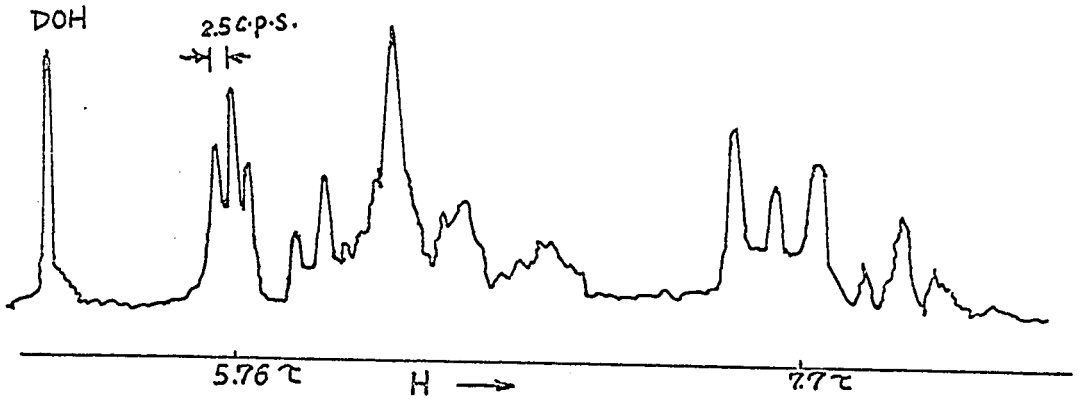


Fig. 11. N.M.R. Spectrum of D,L-1α,3α-diamino-4α, 5α,6β-trihydroxycyclohexane (XVI) dihydrochloride in deuterium oxide.

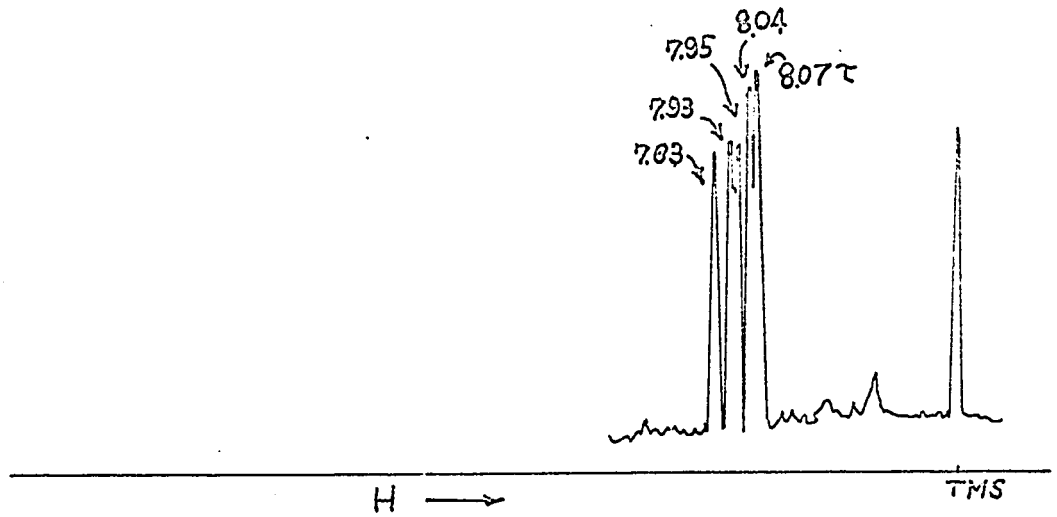
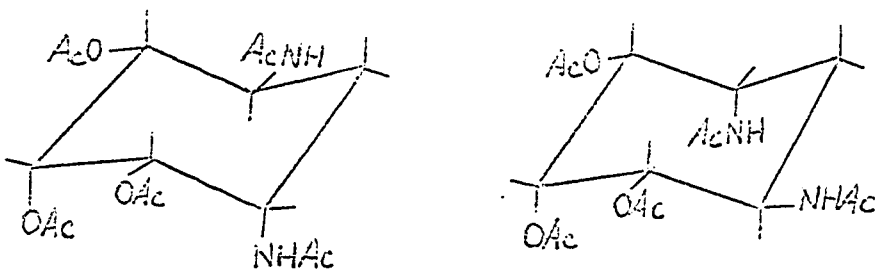


Fig. 12. N.M.R Spectrum of D,L-1α,3α-diacetamido-4α, 5α,6β-triacetoxycyclohexane (XXII) in deuterochloroform.

(3) D,L-1α,3β-Diamino-4α,5α,6α-trihydroxycyclohexane (XVII)

The n.m.r. spectrum of the aminocyclitol R_{ds} 0.96 (dihydrochloride) in deuterium oxide is given in Fig. 13. A well-resolved triplet of intensity one was found at lower field (5.79τ). This triplet was assigned to a hydrogen (at C-4 or C-6) located vicinal to one of the amino groups (cf. the deshielding effect mentioned under (1)). Signals of the hydrogen vicinal to the other amino group seemed to be grouped together with other signals at higher field. The difference in chemical shift of these two structurally equivalent hydrogens at C-4 and C-6 showed that the molecule is not symmetrical. The spacing of 3.0 c.p.s. of the triplet required the proton to be (i) equatorial and vicinal to two axial protons (ii) axial and vicinal to two equatorial protons (iii) equatorial and vicinal to two equatorial protons or (iv) equatorial and vicinal to one equatorial and one axial protons. Such possibilities can give rise to five partial formulas as mentioned above (page 72a). The complete formulas derived from these partial formulas total fifteen; however, only six of them ($\underline{a}_1$, $\underline{a}_3$, $\underline{b}_1$, $\underline{c}_2$, $\underline{d}_1$ and $\underline{e}_1$) are independent (page 73a).

The spectrum of the pentaacetate XXIII (Fig. 14) revealed the presence of only one <u>axial</u> acetoxyl group (7.70 τ), and the signal at highest field (7.96 τ) was assigned to an <u>equatorial</u> acetamido group. The formula of $\underline{c}_2$ or $\underline{e}_1$ cannot provide the data obtained from the pentaacetate spectrum and was therefore rejected. Since the periodate data (p. 65) in this case indicated a <u>cis-cis</u> glycol grouping, formulas $\underline{a}_1$, $\underline{a}_3$ and $\underline{d}_1$ are presumably eliminated, and formula $\underline{b}_1$ is accepted. The aminocyclitol of R_{ds} 0.96 is likely D,L-1α,3β-diamino-4α,5α,6α-trihydroxycyclohexane (XVII).



XXIII

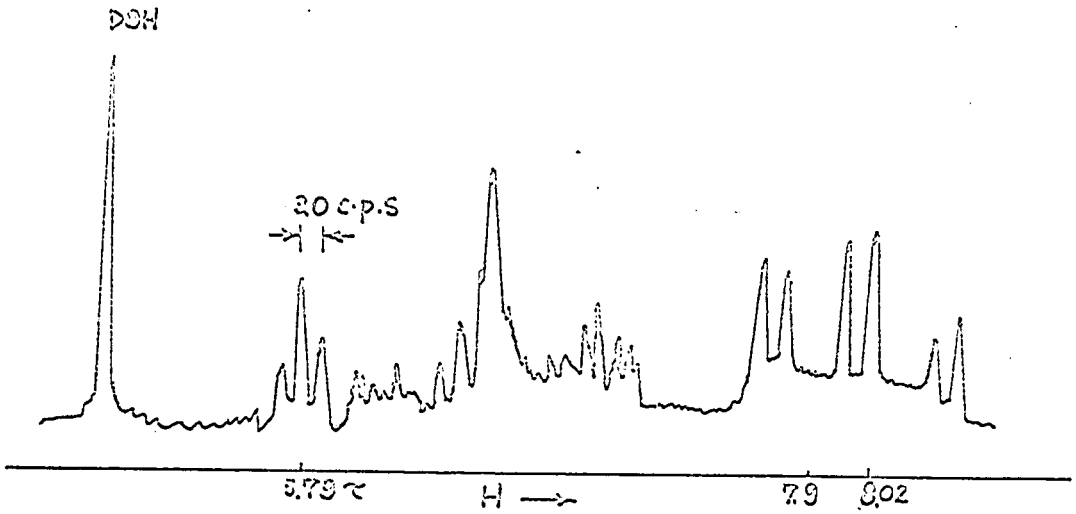


Fig. 13. N.M.R. Spectrum of D,L-1α,3β-diamino-4α,5α,6α-trihydroxycyclohexane (XVII) dihydrochloride in deuterium oxide.

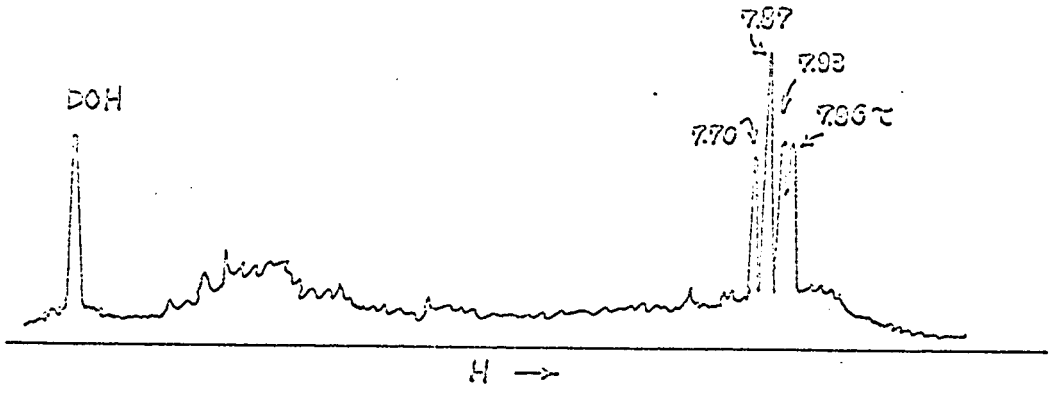
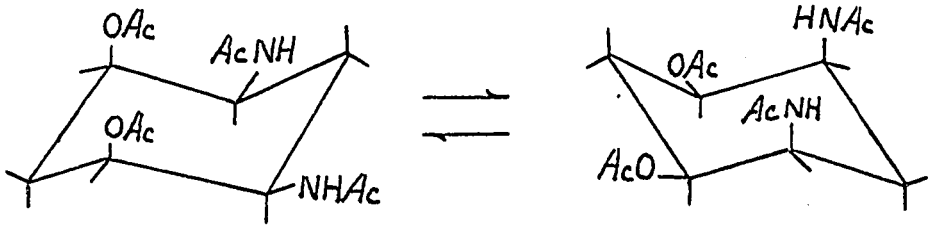


Fig. 14. N.M.R. Spectrum of D,L-1α,3β-diacetamido-4α,5α,6α-triacetoxycyclohexane (XXIII) in deuterium oxide.

(4) 1α,3α-Diamino-4α,6α-dihydroxycyclohexane (XVIII)

The n.m.r. spectrum of the N,N'-diacetate of the aminocyclitol R_{ds} 1.15 was taken in deuterium oxide. Only one sharp signal at 7.89 τ was found for the two acetamido groups at C-1 and C-3. This suggested the possibility that the N,N'-diacetate is built symmetrically, the two acetamido groups being cis to each other and the two hydroxyl groups at C-4 and C-6 likewise being cis to each other.

The n.m.r. spectrum of the water-soluble tetraacetate XXV of the aminocyclitol in deuterium oxide is shown in Fig.15. Two blunt signals of equal intensities appeared at 7.80 and 7.96 τ. The former signal was assigned to two axial acetoxyl groups and the latter to two equatorial acetamido groups. A likely explanation for the bluntness of the signals might be that the tetraacetate XXV shows conformational instability, with an appreciable amount of the inverted chair form in equilibrium.



XXV

It was concluded that the aminocyclitol R_{ds} 1.15 was 1α,3α-diamino-4α,6α-dihydroxycyclohexane (XVIII).

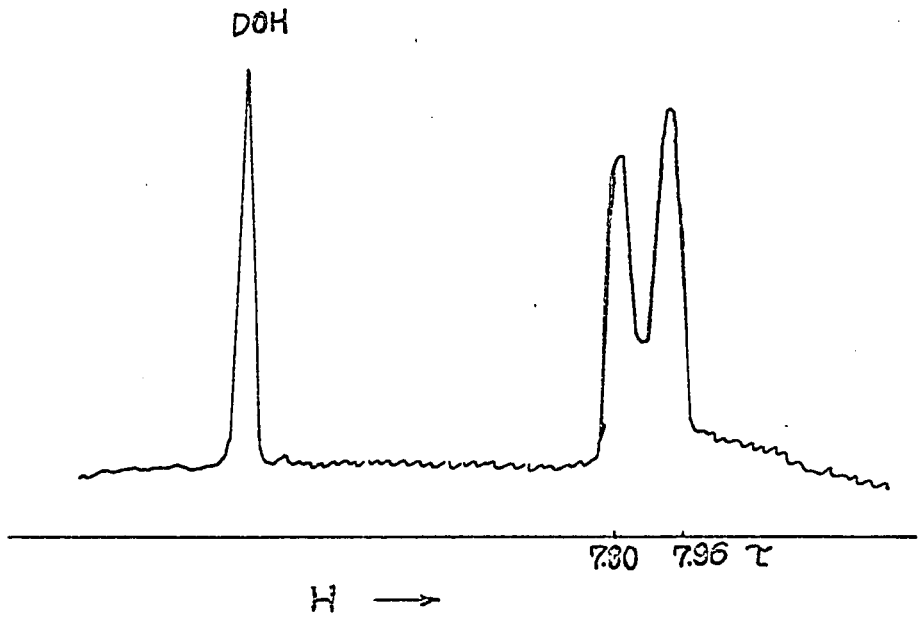
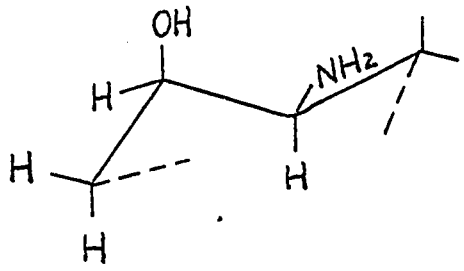


Fig. 15. N.M.R. Spectrum of the water-soluble 1α,3α-diacetamido-4α,6α-diacetoxycyclohexane (XXV) in deuterium oxide.

(5) D,L-1α,3α-Diamino-4α,6β- dihydroxyclohexane (XIX)

The n.m.r. spectrum of compound R_{ds} 1.35 dihydrochloride in D_2O is depicted in Fig. 16. Whereas most proton signals were poorly resolved, there occurred a triplet of intensity one at lowest field (5.75τ). This triplet was assigned to a hydrogen, at C-4 or C-6, vicinal to an amino group (58, 62). Its small splitting of 2.5 c.p.s. indicated that the hydrogen was equatorially oriented and coupled with two axial protons. Apparently, splitting by the vicinal, equatorial methylene proton was too small to be seen. Had the hydrogen that gave the 5.75τ-signal been an axial one, a large splitting should have resulted from the axial methylene proton, and the character of the signal should have been that of an octet rather than a triplet. From this follows the partial formula f.



f

The partial structure f of the aminocyclitol was completed by the n.m.r. spectrum of the tetraacetate in deuterochloroform. The spectrum showed three signals of intensities corresponding

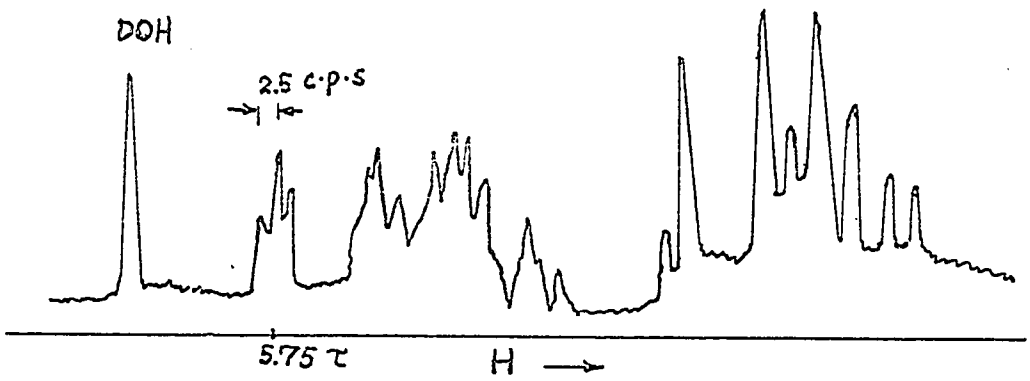
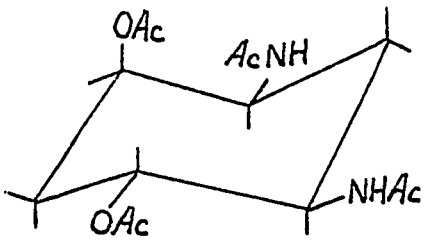


Fig. 16. N.M.R. Spectrum of D,L-1α,3α-diamino-4α,6β-dihydroxycyclohexane (XIX) in deuterium oxide.

to about six, three and three protons at 8.03, 7.96 and 7.83 τ, respectively. The signal of 7.83 τ was assigned to an axial acetoxyl group and that of 7.96 τ to an equatorial acetoxyl group (127-129). The signal of 8.03 τ was assigned, accordingly, to two equatorial acetamido groups. Thus, the tetraacetate can be represented by formula XXVI. Since this formula shows asymmetry, the compound, which was made from symmetrical material must be a racemate.

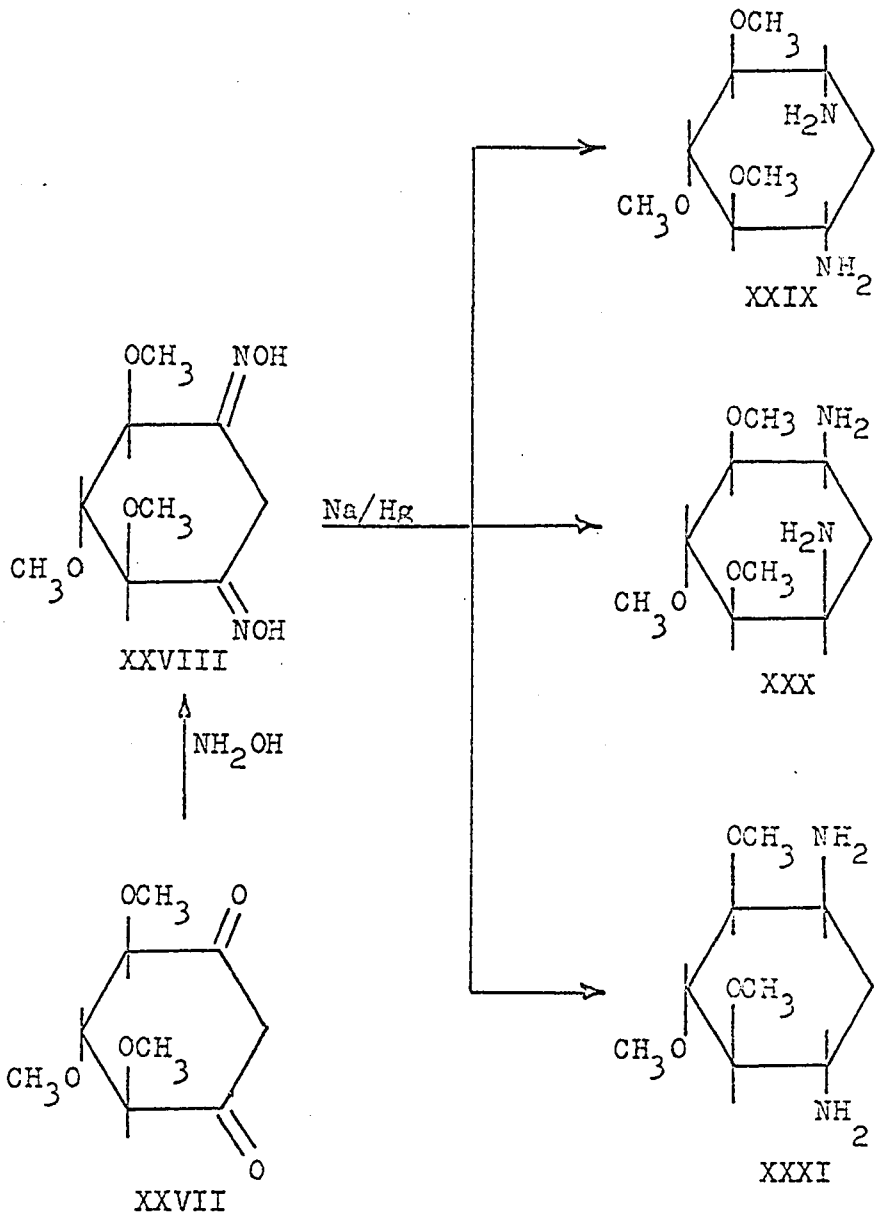


XXVI

It was concluded that compound R_{ds} 1.35 was D,L-1α,3α-diamino-4α,6β-dihydroxycyclohexane (XIX).

Part II Attempted Stereospecific Synthesis of 2-Deoxystreptamine

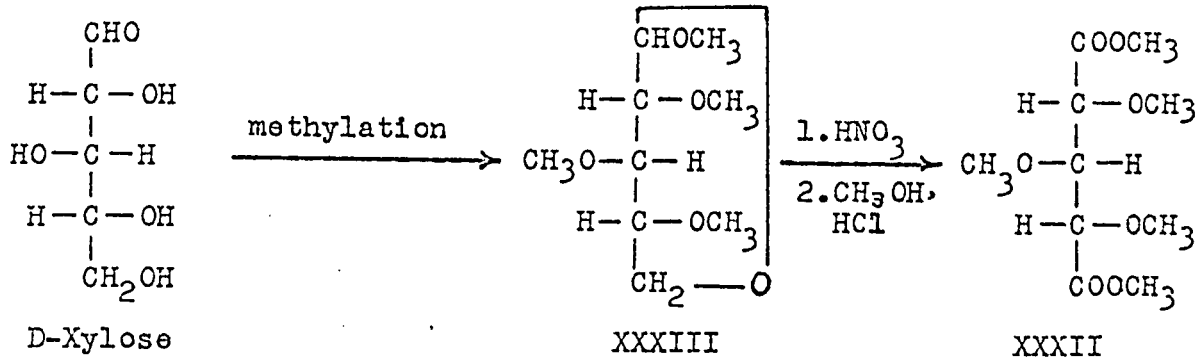
In order to perform a more stereoselective synthesis of 2-deoxystreptamine (XI), D-xylose was chosen as starting material because it has the required, three contiguous trans hydroxyl groups. It was planned that a suitable derivative of this five-carbon sugar and a source that would provide the sixth carbon atom be condensed so as to furnish trans,trans-4,5,6-trihydroxy-1,3-cyclohexanedione or a derivative thereof. Alternatively, chain extension of a xylose derivative followed by cyclization could be pursued. It was decided in these syntheses to protect the secondary hydroxyl groups by methylation; the intermediate to be so produced is trans-trans-4,5,6-trimethoxy-1,3-cyclohexanedione (XXVII). Sodium amalgam reduction of its dioxime (XXVIII) is expected to give tri-O-methyl-2-deoxystreptamine (XXIX). Possibly the two stereoisomers, XXX and XXXI, may occur as by-products in such reduction, but sodium amalgam is known to place an amino group that arises out of an oxime mainly into equatorial position (20,72). The attempted syntheses of the diketone XXVII are now described.


OCH3
H2N
CH3O
OCH3
NH2
XXIX
OCH3 NOH
OCH3
CH3O
NOH
XXVIII
Na/Hg
OCH3 NH2
H2N
CH3O
OCH3
XXX
NH2OH
OCH3 NH2
OCH3
CH3O
NH2
XXXI
OCH3 O
OCH3
CH3O
O
XXVII

1. Dimethyl 2,3,4-Tri-O-methyl-xylarate (XXXII)

Dimethyl 2,3,4-tri-O-methyl-xylarate (XXXII) (130) was prepared by oxidation (131) of methyl 2,3,4-tri-O-methyl-D-xylopyranosides (XXXIII), the latter being obtained from D-xylose by methylation (132, 133). In order to find out whether our preparation of XXXII might be contaminated with impurities, an n.m.r. spectrum was taken (Fig. 17).

A signal of intensity six at 6.23 τ was assigned to the two carbomethoxy groups based on the fact that carboalkoxy groups usually appear at lower field than alkoxy groups (134). A signal of intensity three at 6.54 τ was assigned to the methoxy group on C-3, and a signal of intensity six at 6.60 τ was assigned to the two methoxy groups on C-2 and C-4. A clearly resolved doublet of intensity two at 6.08 τ was assigned to the two hydrogens on C-2 and C-4. The spacing, 2.5 c.p.s. of the doublet was attributed to the coupling with the hydrogen on C-3 . The signal of intensity one for the hydrogen on C-3 appeared at 6.43 τ. The coupling of this hydrogen with the two hydrogens on C-2 and C-4 was not prominent due to the partial overlapping of the signal with that of the methoxy group on C-3. The spectrum appeared to be straightforward and did not reveal any inhomogeneity in the preparation.



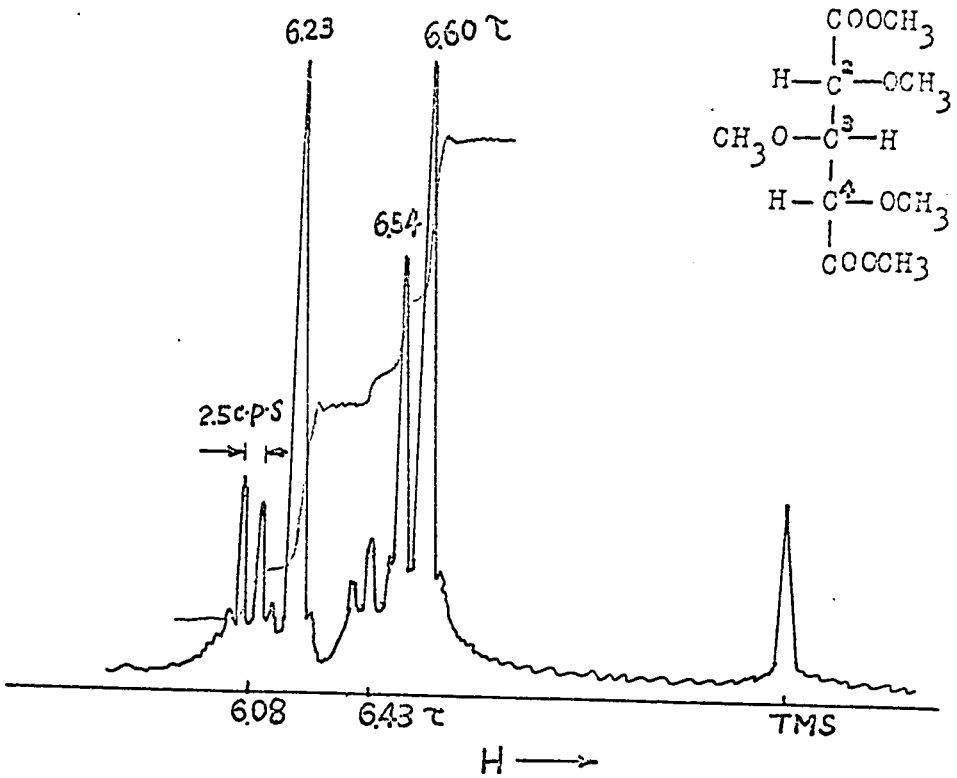
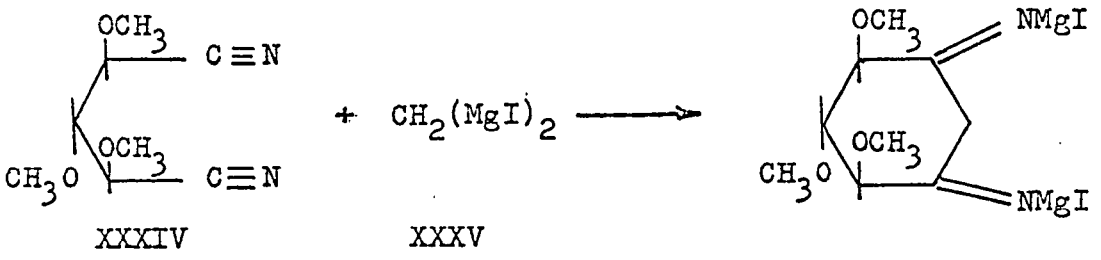


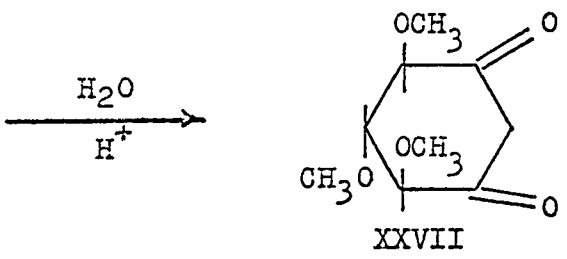
Fig. 17. N.M.R. Spectrum of dimethyl 2,3,4-tri-O-methyl-xylarate (XXXII) in deuterochloroform.

2. Preliminary Attempt at Cyclization

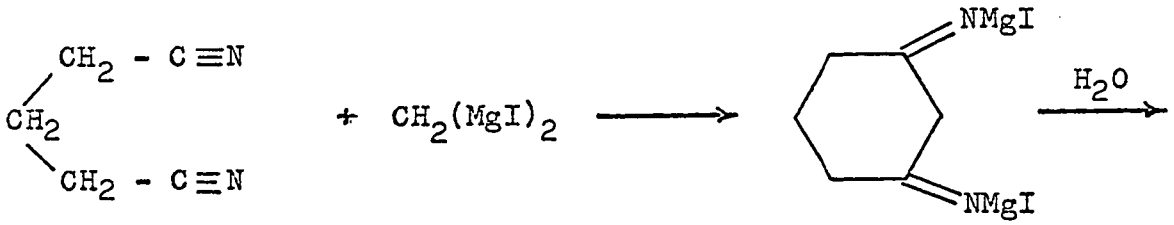
A. It is well-known that a reaction between a nitrile and a Grignard reagent yields, under normal conditions, a ketimine or a ketone (135). If the same type of Grignard reaction could be applied to 2,3,4-tri-O-methyl-xylaronitrile (XXXIV) using methylene dimagnesium diiodide (XXXV), it might be expected that the diketone XXVII would be obtained.



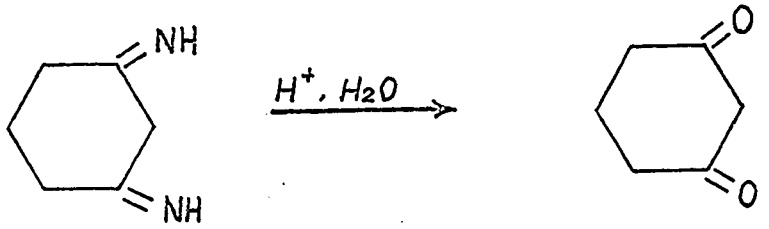
XXXIV XXXV



XXVII



glutaronitrile



1,3-cyclohexanediimine 1,3-cyclohexanedione

$$CH_2(MgI)_2 + 2H_2O \longrightarrow CH_4 + 2MgI(OH)$$

XXXV — methane

$$CH_2(MgI)_2 + \begin{matrix} ClCOOC_2H_5 \\ ClCOOC_2H_5 \end{matrix} \longrightarrow CH_2(COOC_2H_5)_2$$

XXXV — ethyl chloroformate — ethyl malonate

A preliminary experiment was carried out in order to study the reaction between methylene dimagnesium diiodide (XXXV) and a model compound, glutaronitrile. It was hoped that the Grignard reaction would lead to the formation of 1,3-cyclohexanediimine or 1,3-cyclohexanedione.

The Grignard reagent XXXV was first prepared by Emschwiller (136) from methylene iodide and magnesium in a yield of 11%. The existence of XXXV was proved by spontaneous evolution of methane on reaction with water. The same author also reported a reaction between this reagent and ethyl chloroformate to give ethyl malonate (137). Recently, Fidler *et al.* (138) improved the yield to 54% in the preparation of the reagent XXXV.

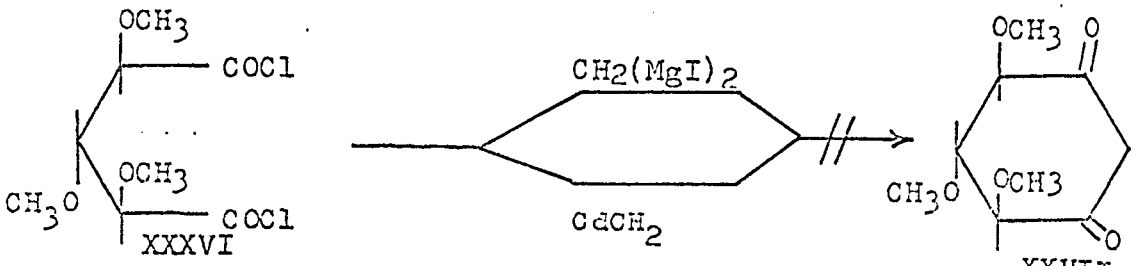
In our work, methylene dimagnesium diiodide (XXXV) was prepared according to Fidler *et al.* (138), and an ether or tetrahydrofuran solution of glutaronitrile was then added. From the reaction mixture most of the unreacted starting material was recovered, in addition to a small amount of resinous product. As shown by its infrared spectrum and a negative ferric chloride test, latter product did not appear to be 1,3-cyclohexanedione or 1,3-cyclohexanediimine.

B. It is known that ketones may be prepared from acyl chlorides and Grignard reagents by dropwise addition of the latter to the former reactant at room temperature or below (139).

In our work, 2,3,4-tri-O-methyl-xylaryl chloride (XXXVI) was prepared from 2,3,4-tri-O-methyl-xylaric acid (XXXVII), the latter being obtained from dimethyl 2,3,4-tri-O-methyl-xylarate (XXXII) according to Hirst and Purves (130). From the reaction mixture of the acyl chloride XXXVI and Grignard reagent XXXV, only the diacid XXXVII was isolated. This indicated that most of the acyl chloride had remained unattacked by the reagent, and subsequent hydrolysis yielded the diacid XXXVII. A possible explanation was the inability of the acyl chloride XXXVI to penetrate the bulky etherate complex (XXXVIII).

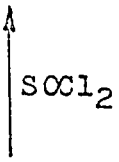
In an attempt to reduce steric hindrance, removal of the solvating ether was considered. This was done by warming the Grignard reagent in a stream of argon gas according to Ziegler (140). The resulting white solid was then mixed with the acyl chloride XXXVI in benzene. However, no ketone formation was obtained. The infrared spectrum of the product that resulted on work-up suggested that 2,3,4-tri-O-methyl-xylaric anhydride was produced.

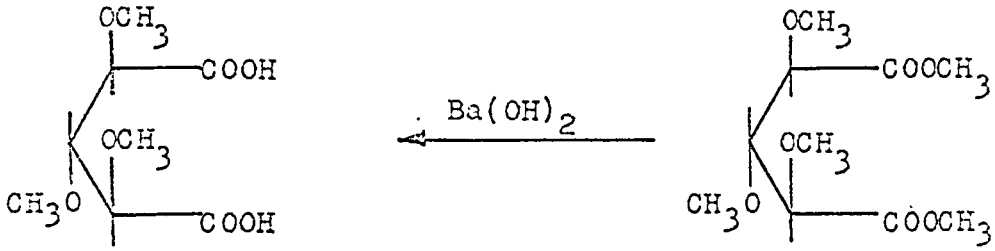
The use of organocadmium compounds for the preparation of ketones from acyl chlorides was first recommended by Gilman and Nelson (141a), and was later applied quite extensively. Organocadmium compounds are readily prepared from the corresponding Grignard reagents and appear to be unreactive towards



XXXVI

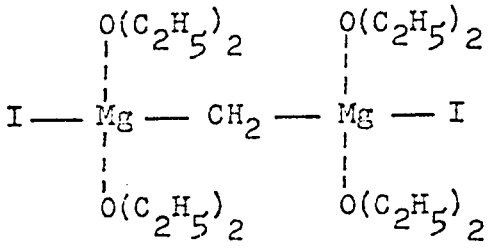
XXVII





XXXVII

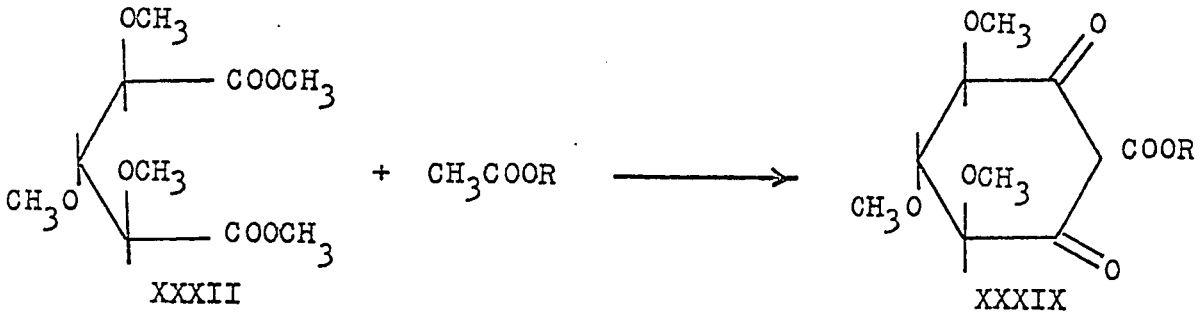
XXXII



XXXVIII

carbonyl groups. In our work, methylene cadmium was prepared from the Grignard reagent XXXV and cadmium chloride (141b), and was reacted with the acyl chloride XXXVI. Again, only trimethylxylaric acid as well as its anhydride were isolated. Thus, the synthesis of the 1,3-cyclohexanedione derivative XXVII using organometallic reagents was not feasible, and this approach was abandoned.

C. Dieckmann cyclization has been successfully employed in the synthesis of 1,3-cyclopentanedione derivatives (142). A similar condensation between the diester XXXII and methyl (or ethyl) acetate gave, however, yellow syrups the properties of which did not correspond to those of an expected 1,3-diketone derivative XXXIX.



XXXII XXXIX

R=-CH_3 or -C_2H_5

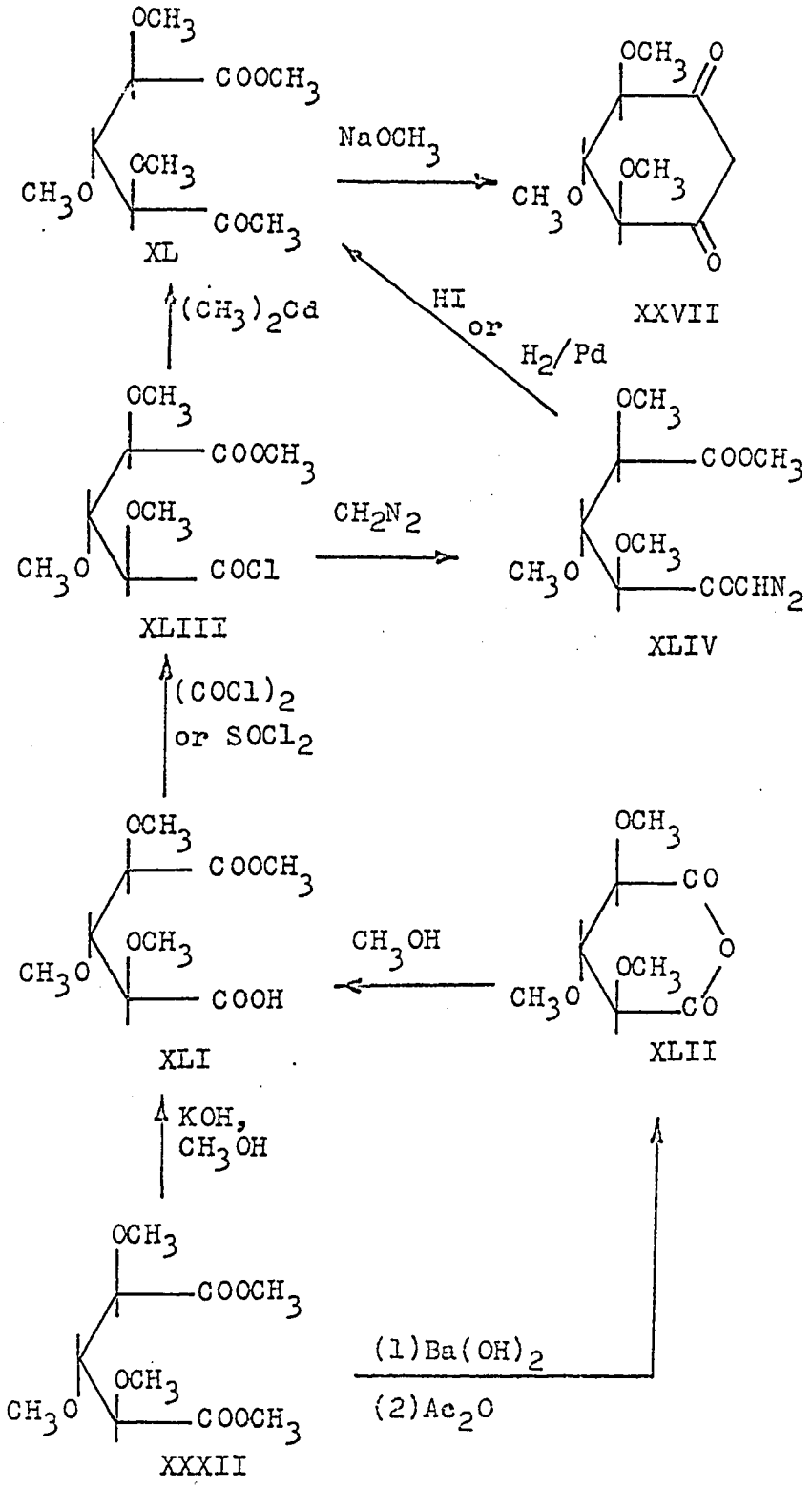
3. Attempted Preparation of Methyl 6-Deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XL)

Since direct cyclization of the diester XXXII or the acyl chloride XXXVI had been unsuccessful, attention was focused on the synthesis of methyl 6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XL) from the diester XXXII. The ketone ester XL would be expected to give the 1,3-cyclohexanedione derivative XXVII when cyclized with sodium methoxide according to Nakazawa (143).

A. Methyl Hydrogen 2,3,4-tri-O-Methyl-D,L-Xylarate (XLI)

Methyl hydrogen 2,3,4-tri-O-methyl-D,L-xylarate (XLI) was obtained in 61% yield by partial hydrolysis of the diester XXXII using the method of Breslow et al. (144). By an alternative method (145), the half ester XLI was prepared from 2,3,4-tri-O-methyl-xylaric anhydride (XLII), the latter being obtained from the diacid XXXVII. The overall yield was 49%. It was found that for the preparation of the half ester XLI, partial hydrolysis of the diester XXXII by alcoholic potassium hydroxide was the superior method.

The half ester XLI was characterized by analysis and infrared spectrum. The n.m.r. spectrum in deuterochloroform is shown in Fig. 18. A signal of intensity three at 6.21 τ was assigned to the carbomethoxy group, as the protons of this group could be expected to resonate at lowest field. Three signals of total intensity nine at 6.51, 6.55 and 6.62 τ were undoubtedly due to the three methoxy groups.


OCH3
COOCH3
CH3O
OCH3
COCH3
XL
NaOCH3
OCH3
O
CH3O
OCH3
O
XXVII
HI or H2/Pd
(CH3)2Cd
OCH3
COOCH3
CH3O
OCH3
COCl
XLIII
CH2N2
OCH3
COOCH3
CH3O
OCH3
CCHN2
XLIV
(COCl)2
or SOCl2
OCH3
COOCH3
CH3O
OCH3
COOH
XLI
CH3OH
OCH3
CO
O
CH3O
OCH3
CO
XLII
KOH,
CH3OH
OCH3
COOCH3
CH3O
OCH3
COOCH3
XXXII
(1)Ba(OH)2
(2)Ac2O

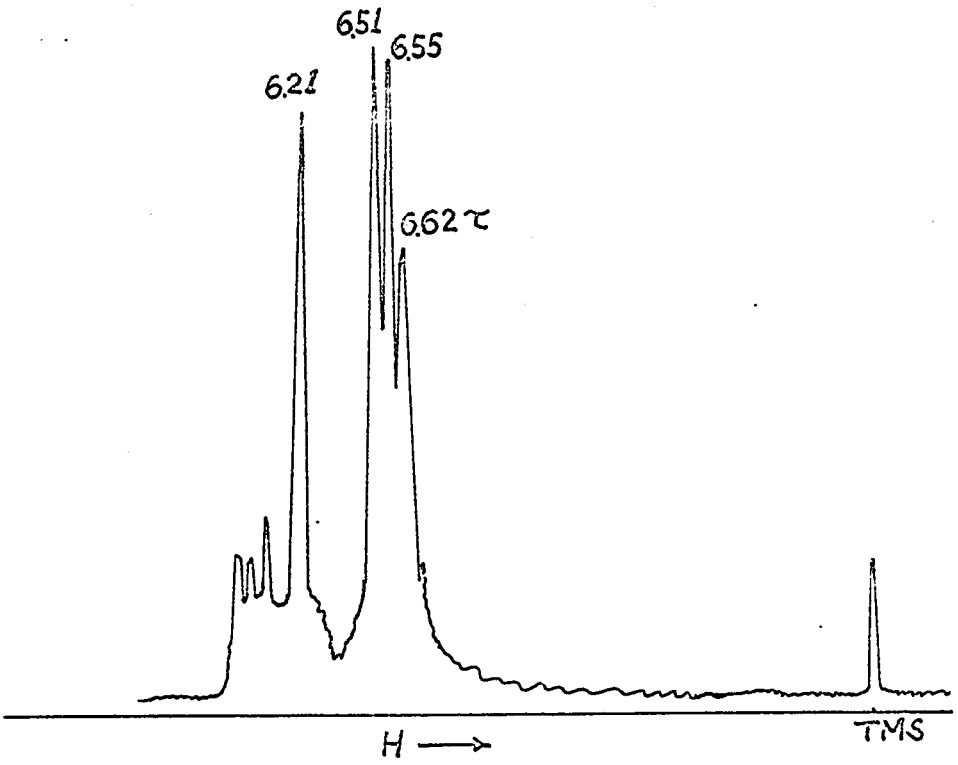


Fig. 18. N.M.R. Spectrum of methyl hydrogen 2,3,4-tri-O-methyl D,L-xylarate (XLI) in deuterochloroform.

B. γ-Carbomethoxy-D,L-xylo-trimethoxy-butyryl Chloride (XLIII)

γ-Carbomethoxy-D,L-xylo-trimethoxy-butyryl chloride (XLIII) was prepared from the half ester XLI using oxalyl chloride (146) (yield,55%) or thionyl chloride (145) (yield,57%). The acyl chloride XLIII was characterized by its analysis, infrared and n.m.r. spectra. The n.m.r. spectrum is shown in Fig. 19. An appearance of a doublet (6.20 and 6.22 τ) of intensity three was quite unexpected because it was anticipated that the compound should give in this region a single signal of intensity three for the carbomethoxy group. Four signals of over-all intensity nine at 6.48, 6.54, 6.57 and 6.62 τ were obviously due to the three methoxy groups. A reason for the observed duplicity of CH_3- signals cannot readily be given unless one were to assume hindrance to free rotation in the molecule to a large enough extent so as to stabilize two different conformations.

C. Methyl 6-Diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV)

Next, it was necessary to convert the acyl chloride XLIII into the corresponding methyl ketone XL. It was decided to try this first by way of the diazoketone, methyl 6-diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV). This diazoketone was prepared from the acyl chloride XLIII and diazomethane by the method of Walker (147). A yellow syrup was obtained, which was characterized by infrared and n.m.r. spectra.

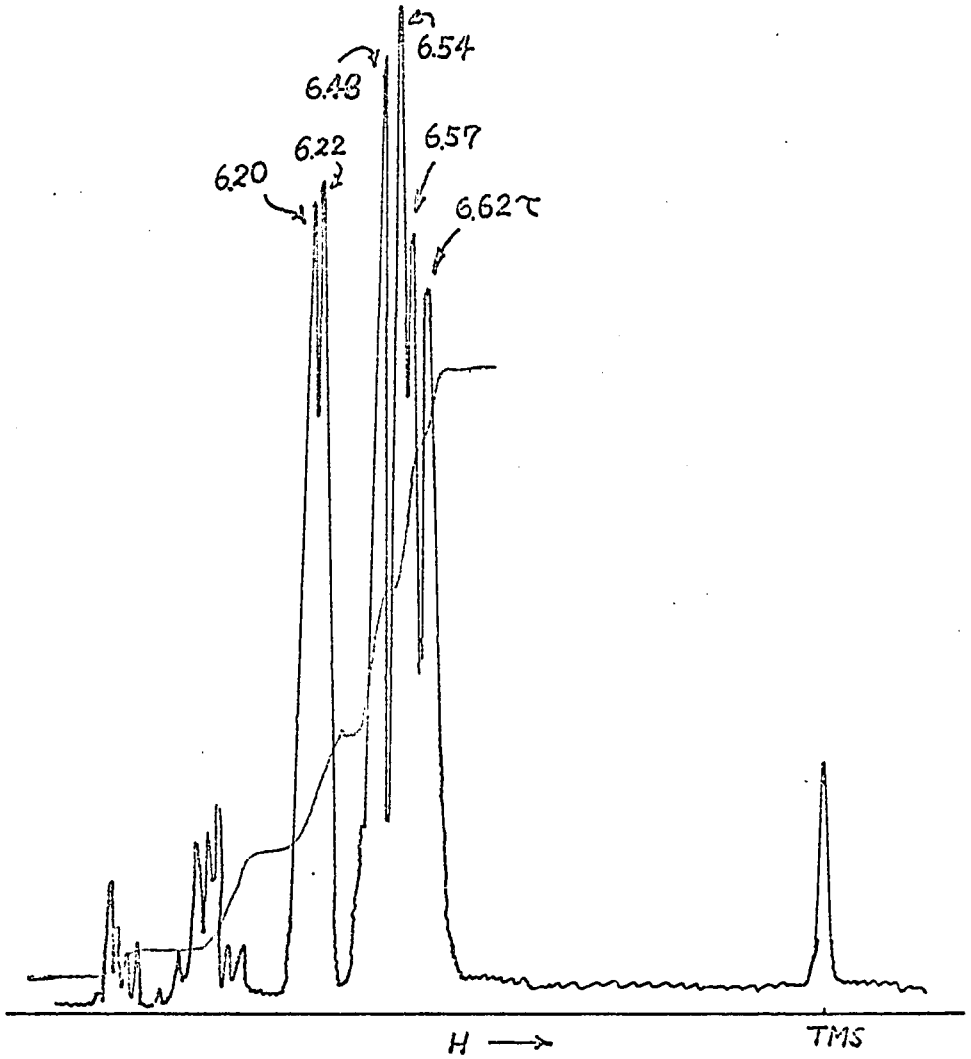


Fig. 19. N.M.R. Spectrum of γ-carbomethoxy-D,L-xylo-trimethoxy-butyryl chloride (XLIII) in deuterochloroform.

The infrared spectrum showed a band at 3100 cm^{-1} due to the C-H bond in $-CO-CH=\overset{+}{N}=\overset{-}{N}$ and two prominent peaks at 2110 and 1630 cm^{-1} which are characteristic of a diazo bond in the forms of $-\underset{H}{\underset{|}{C}}=N\equiv N$ and $-\underset{H}{\underset{|}{C}}=\overset{+}{N}=\overset{-}{N}$, respectively.

The n.m.r. spectrum in deuterochloroform is shown in Fig. 20. A signal appeared at 6.23τ, which was expected for the carbomethoxy group. However, the enhanced intensity of the signal indicated a possible overlapping with that of the hydrogens on C-2, C-3 or C-4. Three signals appeared at 6.54, 6.56 and 6.59τwhich were most likely due to the methoxy groups.

D. Attempted Reduction of Methyl 6-Diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) with Hydriodic Acid

A reaction of methyl 6-diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) with hydriodic acid according to the method of Wolfrom and Brown (148) should have given methyl 6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XL). However, combustion analysis and n.m.r. spectrum of the product showed that this had not occured. The empirical formula indicated the product to be $C_{12}H_{20}O_9$ instead of the expected $C_{10}H_{18}O_6$ (XL).

The infrared spectrum showed bands for methoxyl and carbomethoxyl groups but no hydroxyl or diazo bands. The reaction product gave a 2,4-dinitrophonylhydrazone as

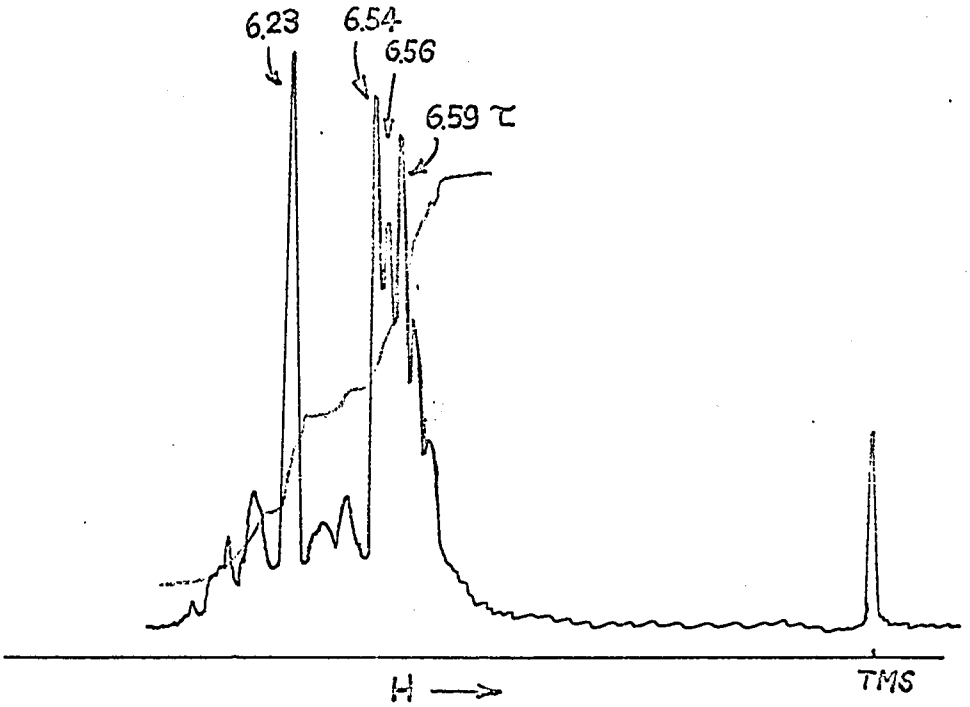


Fig. 20. N.M.R. Spectrum of methyl 6-diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) in deuterochloroform.

brick-red crystals. On heating, the 2,4-dinitrophenylhydrazone decomposed over a wide range 100-140°.

The n.m.r. spectrum of the product $C_{12}H_{20}O_9$ in deuterochloroform (Fig. 21) permitted no simple interpretation.

E. Catalytic Hydrogenation of Methyl 6-Diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) with Palladium on Charcoal

When methyl 6-diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) was hydrogenated in the presence of 10% palladium on charcoal, the diazo group was removed, but the product obtained was not the expected ketonic ester XL. This was evident from the n.m.r. spectrum (Fig. 22); had the product been the ketonic ester XL, the spectrum should have shown a methyl ketone signal of intensity three near the region of 8τ. The structure of the spectrum was similar to that of the product from the hydriodic acid reduction (Fig. 21), except that no signal of 6.34τ was present.

F. Reaction of τ-Carbomethoxy-D,L-xylo-trimethoxybutyryl Chloride (XLIII) with Dimethyl Cadmium

A reaction of τ-carbomethoxy-D,L-xylo-trimethoxybutyryl chloride (XLIII) and dimethyl cadmium (141b) was tried in a further attempt to prepare the ketonic ester XL. The resulting colorless oil had an empirical formula of $C_{10}H_{20}O_6$ instead of the expected $C_{10}H_{18}O_6$ (XL). There is a possibility that the reaction had gone past the stage of ketone

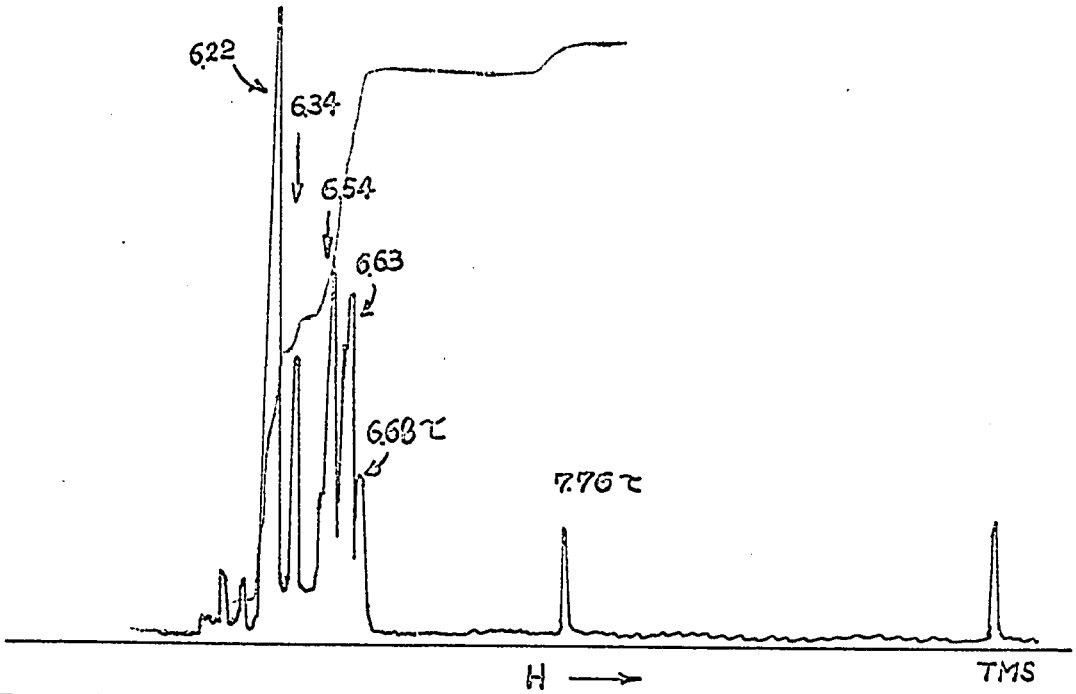


Fig. 21. N.M.R. Spectrum in deuterochloroform of the product obtained from methyl-6-diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) and hydriodic acid.

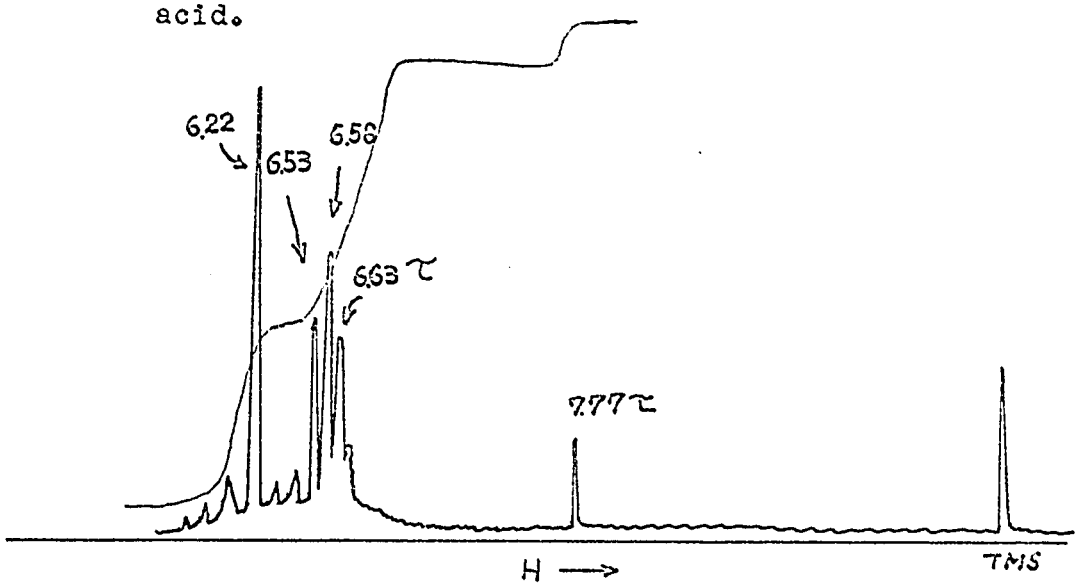
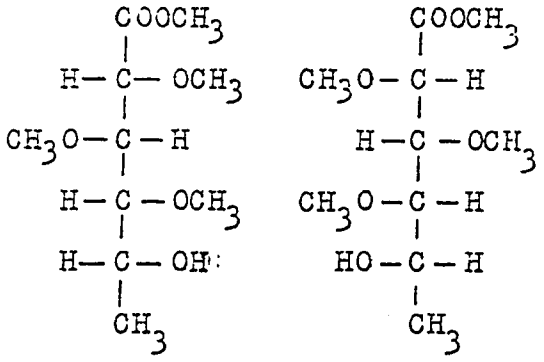
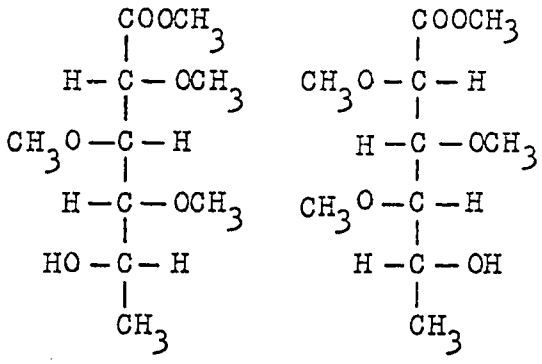


Fig. 22. N.M.R. Spectrum in deuterochloroform of the product obtained from catalytic hydrogenation of methyl-6-diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) in the presence of 10% palladium on charcoal.

formation and that the product consisted of a mixture of methyl 6-deoxy-2,3,4-tri-O-methyl-D,L-gluconate (XLV) and methyl 6-deoxy-2,3,4-tri-O-methyl-D,L-idonate (XLVI). An analogous production of secondary alcohols has been reported previously (149), although this was in a Grignard and not in a alkyl cadmium reaction. The n.m.r. spectrum (Fig. 23) appeared to support the presence of epimeric esters such as XLV and XLVI in the product. Two doublets of a total intensity of three at 8.77 and 8.86 τ could be assigned to the terminal methyl group (C-6) in XLV and XLVI, and their spacings of 3 c.p.s. could be due to coupling with the hydrogens on C-5. However, it was found that the infrared spectrum did not show any bands between 3000-3635 cm^{-1} for a secondary hydroxyl group on C-5.



XLV



XLVI

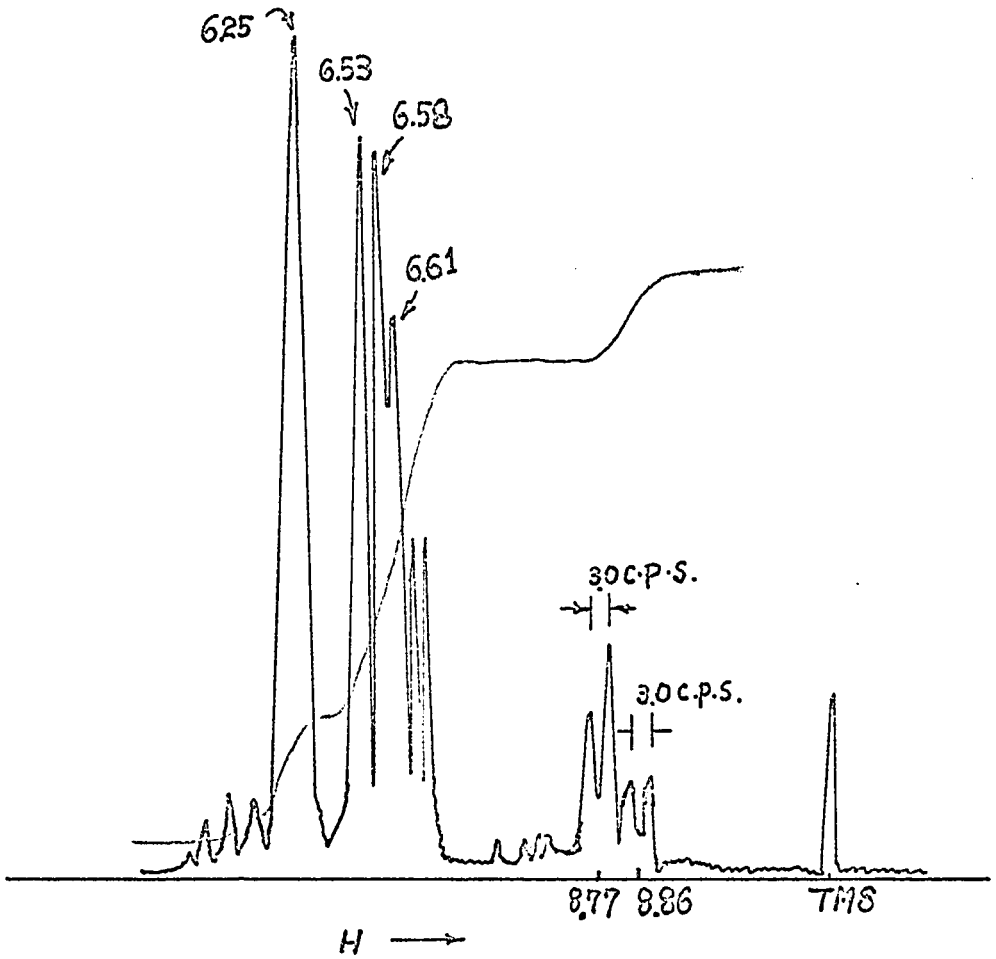


Fig. 23. N.M.R. Spectrum of the product in deuterochloroform obtained from γ-carbomethoxy-D,L-xylo-trimethoxy-butyryl chloride (XLIII) and dimethyl cadmium.

EXPERIMENTAL

The melting points were determined in an aluminium block apparatus and are uncorrected. All evaporations were done *in vacuo* at 35-40° (bath temperature) unless otherwise indicated. Paper chromatography was performed by the descending technique on Whatman No. 1 paper. The aminocyclitols were irrigated, either with pyridine-ethyl acetate-acetic acid-water, 5:5:1:3, v/v (115), or with a borax solvent system. The latter consisted of *n*-butanol-ethanol-borax buffer, 1:1:1, v/v, the buffer containing 9.54 g of borax per liter of water. The paper used with this system was impregnated before with the buffer and dried at room temperature. The spots were indicated with ninhydrin solution (0.6 g of triketohydrindene hydrate, 175 ml of butanol, 15 ml of 2 N acetic acid and 300 ml of methanol). The R_{ds} values are speeds relative to 2-deoxystreptamine (p.52). Infrared spectra were measured using the Nujol mull technique (unless otherwise indicated) and a Perkin-Elmer Infracord instrument. The proton magnetic resonance spectra were measured with a Varian V-4302 spectrometer operating at 60 Mc/sec. When spectra were taken in deuterochloroform they were calibrated with reference to internal tetramethylsilane by the side-band technique. However, they were calibrated with reference to external tetramethylsilane as well as residual DOH when taken in deuterium oxide. The powder x-ray diffraction diagrams were taken using a Philips x-ray diffraction unit. The x-ray source was copper K_α, and the films were exposed for 2 hours.

Part I. Aminocyclitols from 4,6-Dinitropyrogallol

Preparation of 4,6-Dinitropyrogallol (I)

4,6-Dinitropyrogallol (I) was made according to Einhorn, Cobliner and Pfeiffer (110). To 160 g of concentrated sulfuric acid that was magnetically stirred and cooled externally by a dry-ice bath, was added 20 g (0.132 mole) of powdered pyrogallol 1,2-carbonate (III) (110). An orange-colored syrup was formed. A mixture of 17 g of fuming nitric acid and 40 g of concentrated sulfuric acid was then added dropwise while the temperature of the reaction mixture was kept at -16°. The orange-colored syrup became very dark red at this stage. Upon completion of the acid addition, which lasted about one hour, the mixture was stirred for another three hours at -15° and then slowly poured into a large beaker containing 5 g of urea and about 700 g of crushed ice. The beaker was warmed on a steam bath and the mixture was stirred occasionally to hasten the evolution of carbon dioxide. After the evolution of carbon dioxide had ceased, the mixture was cooled. The yellow crystals were filtered off, washed with water and dried in a desiccator. The crude product (22 g) melted at 208-212°. It was recrystallized from aqueous ethanol to afford yellowish needles melting at 214-215°(yield, 78.5%).

Infrared data (in cm^{-1}): phenolic hydroxyl, 3300 (broad), 1060 (strong). Aromatic nitro, 1560 (strong), 1300 (strong). Others, 1630, 1230, 1155, 900, 885, 810, 750, 710.

N.m.r. spectra in three different solvents; acetone, dimethyl formamide and dimethyl sulfoxide, were taken, tetramethylsilane being used as internal reference. In all cases there

was observed only a single, sharp proton signal at 1.52, 1.65 and 1.75 τ respectively.

A similar experiment was done in which the reaction mixture was hydrolyzed at an early stage of nitration, i.e., 30 min. after completion of addition of the acid mixture. A crystalline product was isolated and was found to consist of 4-nitropyrogallol (VIII) which melted at 160°(reported, 162°(110)).

Catalytic Hydrogenation of 4,6-Dinitropyrogallol (I) with Palladium on Charcoal

Palladium on charcoal (1.5 g, 10% Pd) was prehydrogenated in 10 ml of N hydrochloric acid and 20 ml of water. Forty ml of hydrogen was taken up. A mixture of 2.0 g of 4,6-dinitropyrogallol (I), 15 ml of N hydrochloric acid and 30 ml of water was then introduced. The hydrogenation was carried out at room temperature and atmospheric pressure. After 1404 ml of hydrogen had been consumed the hydrogenation became so slow that only 25 ml of hydrogen was absorbed during the next 20 hours despite the addition of fresh catalyst which had been prehydrogenated. The catalyst was filtered off and the filtrate, which became blue-violet on exposure to the air, was concentrated. Ethanol was added to precipitate a deeply colored mass which was found to consist mainly of 4,6-diamino-pyrogallol (IX) dihydrochloride. A reference sample of IX was available (see next paragraph), and comparison was made of the infrared spectra. The observed resistance of IX to further

catalytic hydrogenation with palladium received confirmation by the following experiment.

Attempted Catalytic Hydrogenation of 4,6-Diaminopyrogallol (IX) with Palladium Black

Palladium black was prepared according to Kuhn, Quadbeck and Röhm (104). 4,6-Diaminopyrogallol (IX) dihydrochloride (2.3 g), which had been prepared by reduction of 4,6-dinitropyrogallol (I) with tin according to Einhorn et al. (110), was converted into its free base by treating a solution of the salt in 25 ml of water with Amberlite IR-45(OH^-) until a pH of 5-6 was reached. Glacial acetic acid (50 ml) was added to the mixture and the resin was filtered off. Hydrogenation with palladium black (1 g prehydrogenated in 25 ml of water) at room temperature and atmospheric pressure resulted in a negligible hydrogen uptake during 24 hours. The catalyst was filtered off and the unchanged IX was recovered from the filtrate as its dihydrochloride.

Catalytic Hydrogenation of 4,6-Dinitropyrogallol (I) with Palladium Black

A mixture of palladium black (500 mg), 10 ml of glacial acetic acid and 10 ml of water was prehydrogenated. 4,6-Dinitropyrogallol (I) (130 mg) was then introduced. The hydrogenation was carried out at room temperature and atmospheric pressure. After 97 ml of hydrogen had been consumed rapidly,

further consumption during the next 20 hours was negligible despite the addition of fresh, prehydrogenated catalyst. The catalyst was filtered off, and a few milliliters of N hydrochloric acid was added to the filtrate which was then concentrated to give, on addition of ethanol, a deeply colored mass. The mass was dried in vacuo and was shown by its infrared spectrum to consist mainly of 4,6-diaminopyrogallol (IX) dihydrochloride.

Catalytic Hydrogenation of 4,6-Dinitropyrogallol (I) with Palladium Oxyhydrate on Barium Sulfate

(a) Palladium oxyhydrate on barium sulfate (113) (500 mg) was prehydrogenated in 30 ml of N/10 sulfuric acid. A mixture of 1.0 g of 4,6-dinitropyrogallol (I) and 70 ml of N/10 sulfuric acid was then introduced. The hydrogenation was carried out at room temperature and atmospheric pressure. The initial reduction (685 ml of H_2) to 4,6-diaminopyrogallol (IX) was completed in 2.5 hours, whereupon more prehydrogenated catalyst (2 g) was added and the hydrogenation continued. Further catalyst batches (2 g) were added whenever the uptake of hydrogen became very slow. The reduction took about two days and 1070 ml of hydrogen (calc. for 9 moles, 1,020 ml) were eventually taken up. The catalyst was removed and washed with hot water, and the filtrate — it became violet when exposed to the air — was treated with activated charcoal. The filtrate was then concentrated to one-third of its original volume and methanol (about 350 ml) was

added; this resulted in the formation of 820 mg of grey crystals. The yield was 68%, calculated as diaminotrihydroxycyclohexane sulfate.

Paper chromatography in the borax system of the above product gave five partially overlapping spots centered at R_{ds} 0.30, 0.46, 0.70, 0.78 and 1.00.

(b) A mixture of 3 g of I, 30 ml of N hydrochloric acid, 100 ml of water and 1 g of prehydrogenated palladium oxyhydrate on barium sulfate was hydrogenated at room temperature and atmospheric pressure. The initial reduction (2020 ml of H_2) to 4,6-diaminopyrogallol (IX) was completed in about one hour. The hydrogenation was continued with more prehydrogenated catalyst (a total of 3 g) being added whenever it became too sluggish. The reduction took about four days; 3108 ml of hydrogen (calc. for 9 moles 3060 ml) was eventually consumed. Work-up as described under (a) yielded 2.1 g of grey crystals. The yield was 64.3%, calculated as diaminotrihydroxycyclohexane dihydrochloride.

Paper chromatography on borax paper of this crude product showed the same spot pattern as that of the product obtained in (a).

The crude grey crystals (2.1 g) were converted to the free base form by treatment in about 50 ml of water with Dowex 1-X2 (OH^-) until the solution showed a negative test with silver nitrate. The filtrate was concentrated to a thin syrup which was carefully transferred to the top of a column (2.2 X 43 cm) of

Dowex 1-X2 (borate) (114). The column was eluted with 0.01 M boric acid and 150 fractions of 3.6 ml were collected at a rate of 15 ml/hr. None of the fractions gave a positive ninhydrin reaction, and the experiment was discontinued.

Catalytic Hydrogenation of 4,6-Dinitropyrogallol (I) with Platinum

(a) In a 500-ml reduction vessel were placed 2 g of platinum dioxide catalyst, 40 ml of glacial acetic acid and 40 ml of water. An amount of 450 ml of hydrogen was taken up to reduce the platinum dioxide. A mixture of 5 g (0.023 mole) of 4,6-dinitropyrogallol (I), 50 ml of glacial acetic acid and 50 ml of water was then added. The hydrogen was admitted to the system at room temperature and atmospheric pressure. The color of the reaction solution changed from reddish-brown to light greenish-yellow when 3545 ml of hydrogen was consumed (2 hours). The hydrogenation was continued but became slower than in the initial period. After 50 hours and a total of 5380 ml of hydrogen (calc. for 9 moles, 5100 ml) was taken up. The catalyst was removed and washed with hot water. A stream of air was passed through the filtrate which became brownish on exposure to the air. After several hours the brownish filtrate was decolorized with activated charcoal and then evaporated to a syrup. The syrup was dissolved in 50 ml of N hydrochloric acid, and the solution was treated with activated charcoal. The colorless filtrate was again

concentrated to a syrup and ethanol was added. The colorless crystals appeared which were collected and dried in a desiccator. The yield was 2.21 g (40.1%). Paper chromatography (borax system) revealed that a large number of compounds had been formed (Fig.5).

(b) Catalytic hydrogenation of I in 1/3 N hydrochloric acid was performed similarly. Platinum oxide (1 g) was prehydrogenated in 10 ml of N hydrochloric acid and 10 ml of water. An amount of 185 ml of hydrogen was taken up. A mixture of 2 g of I, 15 ml of N hydrochloric acid and 40 ml of water was then introduced. Hydrogenation under standard conditions resulted in a fast uptake of hydrogen (1350 ml) followed by slow uptake to a total of 2390 ml within 15 hours. Work-up as described in (a) gave 2.7 g of grey crystals. The material was recrystallized from methanol-ethanol to afford 1.0 g of colorless crystals; yield, 46%. Chromatography on borax paper of this product applied in the free base form showed that it consisted of a great number of fast-running substances (Fig. 5).

(c) Hydrogenation of I in 0.23 N sulfuric acid was performed similarly. Platinum oxide (1 g) was prehydrogenated in 30 ml of water; 200 ml of hydrogen was taken up. A mixture of 1.5 g of I, 15 ml of N sulfuric acid and 20 ml of water was then introduced. The hydrogenation was carried out at room temperature and atmospheric pressure and resulted in an uptake of 1540 ml (calc., 1530 ml) of hydrogen within 13 hours. The catalyst was filtered off and 100 ml of methanol was added to yield 500 mg of light yellowish crystals.

Paper chromatography on borax paper (Fig. 5) showed that the pattern of reaction products was quite similar to that obtained (a) in the hydrogenation in acetic acid.

Fractional Recrystallization and Chromatography on Cellulose Powder

Crude hydrogenation product obtained by use of platinum catalyst in 50% acetic acid was fractionally recrystallized from ethanol-methanol. Although partial separation was effected, each of the five fractions obtained was revealed by paper chromatography to consist of at least three components, and some of the components were distributed over several of the fractions. This approach at separation was, therefore, abandoned.

For column chromatography on cellulose, crude product (0.5 g) from the Pt-catalyzed hydrogenation in acetic acid was converted to the free base form by treatment with Dowex 1-X2 (OH^-) in water. The filtrate was concentrated and then transferred onto a cellulose-powder column (2.2 × 38 cm). Elution, with acetone-water (4:1, v/v) did not effect separations.

An identical chromatography was performed using pyridine-ethyl acetate-water-acetic acid (5:5:3:1, v/v) for elution. The flow rate was 15 ml/hr. The 5-ml-fractions 94-124 contained the major components. Although a partial separation was effected, the fractions still were mixtures of several components.

Chromatography on Dowex-50 (H^+) Ion Exchange Resin

A column of Dowex-50(H^+) ion exchange resin (200-400 mesh) was prepared according to Gardell (120). The gradient elution method described by Horowitz, Roseman and Blumenthal (116) was adopted in simplified form. Crude hydrogenation product (3.2 g, from a run in acetic acid)was dissolved in 50 ml of water and carefully transferred onto the column (4.2 × 58 cm). The column was eluted starting with 2 N hydrochloric acid. The fractions were collected in test tubes by an automatic collector. The flow rate was 48 ml/hr. (each tube, 16 ml).

After 110 fractions had been eluted with 2 N acid, gradient elution was started by allowing the reservoir (800 ml 2 N HCl) to be continuously replenished, with magnetic stirring, by 3 N hydrochloric acid.

A total of 450 fractions were collected and were examined by chromatography on borax paper. Fractions were pooled according to their chromatographic patterns. They were blank up to No. 122.

Fractions 123-148.- The combined fractions were evaporated to dryness. The residue was dissolved in 20 ml of water and treated with activated charcoal. The filtered solution was concentrated to a syrup, and 20 ml of ethanol was added. It gave 297 mg of colorless crystals which were identified as ammonium chloride by an infrared spectrum and by evolution of ammonia with cold sodium hydroxide solution. The recovery was 9.3%, based on the amount of material put on the column.

Fractions 199-207.- It was found, by paper chromatography, that these fractions contained practically a pure compound of R_{ds} 0.80. The combined fractions were evaporated to dryness. The residue was dissolved in 20 ml of water and the solution was treated with activated charcoal. The filtered solution was concentrated to a syrup and 5 ml of methanol was added. The mixture was brought to boiling on a steam-bath and ethanol was dropped in to beginning cloudiness. After cooling, 52 mg of colorless crystals were obtained.

Fractions 192-198 and 208-212.- These fractions were found, by paper chromatography, to contain mixtures of two compounds of R_{ds} 0.80 and 0.96. The dry weight was 108 mg. The compound of R_{ds} 0.80 could be isolated in pure form from these fractions by using column chromatography on Dowex 1-X2 (OH^-). The details will be discussed in a later section.

Fractions 219-227.- These fractions yielded 24 mg of colorless crystals which were chromatographically pure compound R_{ds} 0.74.

Fractions 213-218 and 228-290.- These fractions contained four components of R_{ds} 0.74, 0.96, 1.15 and 1.40. The combined fractions were concentrated to a syrup and ethanol was added, to give crystals which, upon recrystallization from methanol-ethanol, weighed 613 mg. The material was found to consist of compounds R_{ds} 0.74 and 0.96 with a trace of compound R_{ds} 1.15. Further separation was achieved on a Dowex-1 (borate) column as described in a subsequent section.

Fractions 320-359.- These fractions afforded 102 mg of colorless crystals of chromatographically pure compound R_{ds} 1.15.

Fractions 361-405.- The combined fractions yielded 62 mg of colorless crystals of chromatographically pure compound R_{ds} 1.35.

Fractions 406-450.- These fractions afforded only 5 mg of a colorless solid which was shown to be a mixture of several compounds.

Chromatography of Fractions 192-198 and 208-212 on Dowex 1-X2 (OH^-) Resin

Dowex 1-X2 (Cl^- form) ion-exchange resin (100-200 mesh) was converted to the hydroxide form before use. It was washed first with 2 N sodium hydroxide (5 bed-volumes) and then with water free from carbon dioxide until the washings were neutral. A column 2.2 X 43 cm was set up. The mixture (108 mg) of compounds R_{ds} 0.80 and 0.96 from fractions 192-198 and 208-212 was converted to the free base form, then applied to the top of the column. Elution was carried out with water free from carbon dioxide at a rate of 8.0 ml/hr. Fractions (each 6.8 ml) were collected in test tubes. The fractions 19-22 still contained, as shown by paper chromatography, a mixture of the two compounds. The fractions 23 and 24 were combined, acidified with dilute hydrochloric acid, and evaporated to dryness. Recrystallization of the residue from methanol-ethyl acetate yielded 20 mg of colorless crystals that were chromatographically pure compound R_{ds} 0.80.

Chromatography of Fractions 213-218 and 228-290 on Dowex 1-X2 (borate) Resin

Dowex 1-X2(Cl^-form) ion-exchange resin (200-400 mesh) was washed with a saturated solution of potassium tetraborate until the washings gave only a faint silver nitrate test for chloride ion. It was then washed with water until the washings showed a negative test for borate ion. The resin was suspended in water, poured into a chromatographic tube and allowed to settle slowly. The dimensions of the column were 2.2 X 43 cm.

The mixture of hydrochlorides (631 mg) of the two compounds R_{ds} 0.74 and 0.96 (with trace of R_{ds} 1.15), obtained from fractions 213-218 and 228-290, was converted to the free base form and then transferred onto the borate column. The column was eluted with 0.01 M boric acid at a rate of 20 ml/hr. Fractions of 7.2 ml were collected in test tubes.

Fractions 10-15 were evaporated to dryness and then evaporated several times with methanol until flame tests for boric acid were negative. The residue was finally acidified with 4 ml of N hydrochloric acid and brought to a syrup. Upon addition to the syrup of methanol and ethanol there separated colorless crystals (237 mg) which were collected and dried in a desiccator. These crystals were chromatographically pure compounds R_{ds} 0.96 dihydrochloride.

Fractions 17-22 from the borate column were evaporated to dryness. Dry methanol was added and the boric acid was removed

as just described. The residue was dissolved in 4 ml of N hydrochloric acid and the solution was evaporated to a syrup. Methanol and ethanol were added to the syrup to yield 246 mg of colorless crystals which consisted of compound R_{ds} 0.74 dihydrochloride in pure state.

1α,3α-Diamino-4α,5α,6α-trihydroxycyclohexane (XV)

The dihydrochloride of the compound with R_{ds} 0.74, as isolated by column chromatography, amounted to 270 mg (=8.4% of the crude hydrogenation product put on the column). When heated on the micro-block, it turned grey at 250° and decomposed at about 290°. A sample for analysis was recrystallized from methanol-ethanol and was dried at 56° in vacuo. The sample for n.m.r. spectrum (Fig. 9) and x-ray diffraction diagram (Fig.7,A) was dried overnight at 100° in vacuo.

Anal. Calcd. for $C_6H_{14}N_2O_3 \cdot 2HCl$ (235.12): C, 30.66; H, 6.86; N, 11.93. Found: C, 30.80; H, 6.76; N, 11.74.

Infrared data (in cm^{-1}).- Hydroxyl, 3300 (strong), 1010 (strong). Amine salt, 1590 (strong), 1490 (strong). Others; 2020, 1140, 1100, 1050, 950, 880 and 785.

The dipicrate of XV was prepared as follows. The dihydrochloride was dissolved in a small amount of water and was converted to the free base with Dowex 1-X2(OH^-). A saturated solution of picric acid in methanol was added to the filtrate until the mixture reacted strongly acidic towards litmus paper. The crystals that separated were collected and recrystallized twice from aqueous

methanol. Although the sample was dried for analysis *in vacuo* at 56°, it appeared to retain solvent of crystallization.

Anal. Calcd. for $C_6H_{14}N_2O_3 \cdot 2C_6H_3N_3O_7 \cdot CH_3OH \cdot H_2O$: C, 34.02 ; H, 3.88; N, 16.72. Found: C, 33.80; H, 3.96; N, 16.85.

The *pentaacetate* XXI of XV was prepared by refluxing, for one hour, 47 mg (0.2 mmole) of the dihydrochloride and 33.4 mg (0.4 mmole) of anhydrous sodium acetate in 3.5 ml of acetic anhydride. The mixture was evaporated to dryness. Water (5 ml) was added to the residue and the mixture was extracted three times with about 10 ml of chloroform. The chloroform extract was dried over anhydrous sodium sulfate. On evaporation, it yielded only a minute amount of colorless crystals insufficient for identification. The aqueous solution left after the chloroform extraaction was treated first with Dowex-50(H^+) to remove sodium ion, then with Amberlite IR-45(OH^-) to remove chloride ion. The filtrate from the resins was evaporated to a syrup. Acetone (10 ml) was added, some insoluble material was filtered off and the solution was concentrated to a syrup which yielded, on addition of a few milliliters of ethanol, 60 mg (95%) of colorless crystals. The pentaacetate melted at 285-287° with decomposition.

Anal. Calcd. for $C_{16}H_{24}N_2O_8$ (372.3): C, 51.60; H, 6.48; N, 7.53. Found: C, 51.59; H, 6.41 ; N, 7.35.

Infrared data (in cm^{-1}).- Amide NH, 3580 (medium), 3275 (strong). Acetoxyl, 1740 (strong). Amide I, 1640 (strong). Amide II, 1535 (strong). Ester, 1220 (strong). Others, 1100, 1050, 1010, 975 and 945.

The n.m.r. spectrum in deuterium oxide is shown in Fig. 10.

D,L-1α,3α-Diamino-4α,5α,6β-trihydroxycyclohexane (XVI)

The crystalline dihydrochloride of the compound with R_{ds} 0.80 amounted to 72 mg; yield was 2.25%. The salt softened at about 180° and decomposed in the range of 250-265° without distinct melting. The sample for analysis was recrystallized from water-ethanol and was dried at 56° in vacuo. The sample for the n.m.r. spectrum (Fig.11) and x-ray diffraction diagram (Fig. 7,B) was dried overnight at 100° in vacuo.

Anal. Calcd. for $C_6H_{14}N_2O_3 \cdot 2HCl \cdot 2H_2O$ (271.15): Cl, 26.16; N, 10.34. Found: Cl, 26.01; N, 10.14.

Infrared data (in cm^{-1}).- Hydroxyl, 3300 (strong), 1050 (strong). Amine salt, 1580 (strong), 1490 (strong). Others; 2000, 1100, 990 and 720.

The pentaacetate XXII of XVI was prepared as follows. A mixture of dihydrochloride (27.1 mg), 2.0 ml of acetic anhydride and 16.7 mg of sodium acetate was refluxed for one hour and evaporated to dryness. Water (5 ml) was added to the residue and the mixture was extracted thrice with chloroform. The chloroform extracts were treated with activated charcoal and dried over anhydrous sodium sulfate. The filtrate, on evaporation, yielded a semi-solid which could not be induced to crystallize. The semi-solid product was used for the n.m.r. spectrum (Fig. 12).

Infrared bands (in cm^{-1}, in chloroform).- Amide NH,

3475 (medium), 3350 (medium). Acetoxyl, 1750 (strong). Amide I, 1675 (strong). Amide II, 1515 (strong). Ester, 1240 (strong). Others; 1090, 1050 and 960.

The aqueous solution left after the chloroform extraction was deionized with Dowex 50(H^+) and Amberlite IR-45(OH^-) resins. Removal of the solvent gave only a trace of substance which could not be identified.

D,L-1α,3β-Diamino-4α,5α,6α-trihydroxycyclohexane (XVII)

The compound of R_{ds} 0.96 isolated as crystalline dihydrochloride amounted to 237 mg; this was a yield of 7.4%. The salt turned grey at 260° and blackened at 295°. The sample for analysis was recrystallized from methanol-ethanol and was dried at 56° *in vacuo*. The sample for the x-ray diffraction diagram (Fig. 7, C) and n.m.r. spectrum (Fig. 13) was dried overnight at 100° *in vacuo*.

Anal. Calcd. for $C_6H_{14}N_2O_3 \cdot 2HCl \cdot \frac{1}{2}C_2H_5OH$ (258.19): C, 32.57; H, 7.42; N, 10.85. Found: C, 32.45; H, 6.83; N, 11.05.

Infrared bands (in cm^{-1}).- Hydroxyl, 3400 (strong), 1020 (strong). Amine salt, 1580 (strong), 1490 (strong), Others; 2000, 1300, 1210, 1090 and 960.

The *N,N'*-diacetate of XVII was prepared as follows. To a sample of 23.5 mg of dihydrochloride dissolved in 1.2 ml of water and 0.15 ml of methanol there was added 0.2 ml of acetic anhydride and 2 ml of Dowex 1-X2 ($CO_3^=$) (118). The mixture was magnetically stirred at room temperature for one hour. The resin

was filtered off and the filtrate was stirred briefly with 1 ml of Dowex 50(H^+). The filtered solution was evaporated to a syrup, and ethanol and ether were added to give 16.5 mg of colorless crystals (Yield, 73.6%). The sample for an n.m.r. spectrum was dried at 56° *in vacuo*. The spectrum in deuterium oxide showed a doublet of equal intensity at 7.90 and 7.92 τ for the two acetamido groups.

Infrared bands (in cm^{-1}).- Hydroxyl, 3300 (strong). Amide I, 1630 (strong). Amide II, 1540 (strong). No acetoxyl carbonyl. Others; 1300, 1145, 1030, 965 and 720.

The *pentaacetate* XXIII of XVII was obtained by refluxing for one hour[a] mixture of 23.5 mg of dihydrochloride, 1.75 ml of acetic anhydride and 16.7 mg of sodium acetae. The mixture was evaporated to a syrup. Water (5 ml) was added to the syrup and the mixture was extracted thrice with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and evaporated, yielding only a trace of residue which could not be identified.

The aqueous solution left after the chloroform extraction was treated first with Dowex 50(H^+) to remove sodium ion, then with Amberlite IR 45(OH^-) to remove chloride ion. Evaporation gave a syrup that could not be induced to crystallize; its n.m.r. spectrum is shown in Fig. 14.

Infrared bands (in chloroform, cm^{-1}).- Amide NH, 3450 (weak). Acetoxyl, 1742 (strong). Amide I, 1675 (strong). Amide II, 1510 (strong). Ester, 1240 (strong). Other; 3000, 2940, 1365, 1285, 1120 and 1040.

1α,3α-Diamino-4α,6α-dihydroxycyclohexane (XVIII)

The compound of R_{ds} 1.15, isolated as crystalline dihydrochloride, amounted to 102 mg, which corresponded to a yield of 3.2%. The salt was recrystallized from methanol-ethanol and dried at 56° in vacuo for 3 hours. The crystals turned grey at 290° but did not melt up to 300°. The sample for the x-ray diffraction diagram (Fig. 7, D) was dried overnight at 100° in vacuo.

Anal. Calcd. for $C_6H_{14}N_2O_2 \cdot 2HCl \cdot \frac{1}{2}CH_3OH$(235.14): C, 33.21; H, 7.71; N, 11.92. Found: C, 33.63; H, 7.47; N, 12.15.
33.79; 7.54;

Infrared bands (in cm^{-1}).- Hydroxyl, 3350 (strong), 1045 (strong). Amine salt 1580 (strong), 1480 (strong). Others; 1940, 1275, 1220, 1160, 1080, 1000, 960 and 815.

N,N'-Diacetate of XVIII:

To a sample of 35.3 mg (0.15 mmole) of dihydrochloride in 1.74 ml of water and 0.23 ml of methanol was added 3 ml of Dowex 1-X2 ($CO_3^=$) and 0.12 ml of acetic anhydride (118). The mixture was stirred magnetically at room temperature for about 90 min., after which time it gave a very faint ninhydrin test. The mixture was filtered and the filtrate was treated briefly with 1 ml of Dowex 50(H^+). The filtrate was then evaporated to a syrup. Ethanol and chloroform were added to the syrup to give 24.7 mg of colorless crystals. The yield was 71.3%. The n.m.r. spectrum in deuterium oxide of these crystals showed a single signal at 7.89 τ for the two acetamido groups.

Infrared bands (in cm^{-1}).- Hydroxyl and amide NH, 3400 (medium), 3340 (medium), and 3220 (strong, broad). Amide I, 1630 (strong). Amide II, 1535 (strong). No acetoxyl carbonyl. Others; 1290, 1270, 1150, 1130, 1090, 980, 965, 940, 835 and 715.

Tetraacetate XXV of XVIII:

A mixture of compound R_{ds} 1.15 dihydrochloride (23.5 mg), 1.5 ml of acetic anhydride and 15 mg of sodium acetate was refluxed for one hour and evaporated to dryness. Water (5 ml) was added to the residue and the mixture was extracted thrice with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and yielded, on evaporation, 3 mg of colorless crystals (Yield, 9.5%). In another run, the same amount of starting material yielded 9 mg of the chloroform-soluble product. The crystals were recrystallized from chloroform-ether and were dried at 56° in vacuo. They decomposed over a range of 230-260°(micro-block).

Anal. Calcd. for $C_{14}H_{22}N_2O_6$ (314.33): C, 53.49; H, 7.06. Found: C, 53.09 ; H, 6.91.

Infrared bands (in cm^{-1}).- Amide NH, 3400 (medium), 3230 (medium). Acetoxyl, 1730 (strong), 1710 (medium). Amide I, 1640 (strong). Amide II, 1530 (strong). Ester, 1250 (strong). Others; 1140, 1090, 1020 and 995.

The aqueous solution left after the chloroform extraction was deionized with Dowex 50(H^+) and Amberlite IR 45(OH^-), and brought to dryness in vacuo. Addition of chloroform furnished

27 mg of colorless crystals (Yield, 85.9%). The sample for analysis and also for the n.m.r. spectrum (Fig. 15) was recrystallized from ethanol-chloroform and dried at 56° in vacuo. The product decomposed over a range of 230-260°.

Anal. Calcd. for $C_{14}H_{22}N_2O_6$ (314.33): C, 53.49; H, 7.06; N, 8.92. Found: C, 53.57; H, 7.08; N, 8.87.

Infrared bands (in cm^{-1}).- Amide NH, 3250 (strong). Acetoxyl, 1730 (strong). Amide I, 1620 (strong). Amide II, 1520 (strong). Ester, 1235 (strong). Others; 1140, 1090, 1020, 990 and 760.

D,L-1α,3α-Diamino-4α,6β-dihydroxycyclohexane (XIX)

The compound of R_{ds} 1.35, isolated as crystalline dihydrochloride, amounted to 62 mg; this was a yield of 1.95%. The substance was recrystallized from water-ethanol and dried at 56° in vacuo; on heating on a micro-block the crystals turned grey at 250° and blackened at about 290°. The n.m.r. spectrum in deuterium oxide is shown in Fig. 16.

Anal. Calcd. for $C_6H_{14}N_2O_2 \cdot 2HCl$ (219.12): Cl, 32.43; N, 12.79. Found: Cl, 33.06; N, 12.84.

Infrared bands (in cm^{-1}).- Hydroxyl, 3370 (strong), 1030 (strong). Amine salt, 1575 (strong), 1490 (strong). Others; 2000, 1285, 1200, 1080 and 960.

Tetraacetate XXVI of XIX:

A mixture of 21.9 mg (0.1 mmole) of dihydrochloride, 1.75 ml of acetic anhydride and 16.7 mg (0.2 mmole) of sodium acetate was refluxed for one hour and evaporated to dryness. Water (5 ml) was added to the residue and the mixture was extracted thrice with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and gave, on evaporation, a residue which could not be induced to crystallize. The n.m.r. spectrum in deuterochloroform showed a signal of 8.03 τ for the two acetamido groups and two signals of 7.96 and 7.83 τ for two acetoxyl groups.

Infrared bands (in syrup, cm^{-1}). Amide NH, 3300 (strong). Acetoxyl, 1730 (strong). Amide I, 1640 (strong). Amide II, 1540 (strong). Ester, 1259 (strong). Others; 1090. 1020, 870 and 800.

Preliminary Experiments for Periodate Oxidations

Preliminary experiments for the selective N-acetylation were carried out with 2-deoxystreptamine and the aminocyclitols XV, XVI, XVII, XVIII, and XIX. A sample of about 0.05 mmole of the aminocyclitol dihydrochloride was weighed and dissolved in 0.7 ml of water and 0.1 ml of methanol in a 25 ml Erlenmeyer flask. Sodium hydrogen carbonate (19 mg) and acetic anhydride (0.03 ml) were added. The mixture was magnetically stirred at room temperature. The progress of N-acetylation was checked by spot tests on filter paper with ninhydrin. The times required for

complete N-acetylation of the six aminocyclitols were found as follows:

2-deoxystreptamine (XI) ; 80 min.
compound R_{ds} 0.74 (XV) ; 90 min.
compound R_{ds} 0.80 (XVI) ; 120 min.
compound R_{ds} 0.96 (XVII); 100 min.
compound R_{ds} 1.15(XVIII); 150 min.
compound R_{ds} 1.35 (XIX) ; 100 min.

Periodate Oxidation

Accurately weighed samples of the aminocyclitol hydrochlorides (about 0.05 mmole) were N-acetylated under the conditions that had been found suitable in the preliminary experiments. The solutions were used for the measurement of periodate consumption, directly without isolation of the N,N'-diacetates. To this end, each solution was transferred into a 25-ml volumetric flask by means of a capillary pipette and with the addition of a few milliliters of aqueous methanol.

The flask was cooled in an ice-water bath. After the content of the flask had attained 3-4°, 5.00 ml of a pre-cooled, standardized sodium metaperiodate solution (0.25 M) was added and the flask was filled to the mark with water of 3-4°, well shaken and restored to the ice-water bath in the dark. At various times, 2-ml aliquots were pipetted into a mixture of 5 ml of M sodium hydrogen carbonate and 2 ml of 20% potassium iodide solution.

The mixture was left 15 minutes in the dark at room temperature and then titrated against 0.03 M sodium arsenite solution. A starch solution (1%) was used as an indicator.

For the periodate oxidation of methyl α-D-glucopyranoside (XII) and methyl α-D-mannopyranoside (XIII), a sample of about 0.05 mmole was weighed accurately and dissolved in a few milliliters of aqueous methanol in a 25-ml volumetric flask. The flask was cooled in an ice-water bath and the oxidation was carried out as described above.

The periodate consumption data are given in Tables III-X and depicted in Figure 8.

Moles of periodate consumed per mole of substance are calculated from the following equation.

$$Y = \frac{C_1 \times \frac{5}{1000} - C_2 \times \frac{V}{1000} \times \frac{25}{2}}{\frac{W}{F}} = \frac{\frac{C_1}{200} - \frac{C_2 \cdot V}{80}}{\frac{W}{F}}$$

Y = moles of periodate consumed per mole of substance

V = volume of arsenite solution consumed in titration (ml) of 2-ml aliquots

C_1 = molarity of periodate solution

C_2 = molarity of arsenite solution

W = weight of sample (g)

F = formula weight of the aminocyclitol

Table III

Periodate Oxidation of N,N'-Diacetate of Compound R_{ds} 0.74 (XV)

The amount of XV-dihydrochloride used for the selective acetylation was 13.25 mg. The concentrations of the sodium metaperiodate solution and the sodium arsenite solution were 0.2446 M and 0.02977 M, respectively.

Time (min.)	Volume (ml) of Sodium Arsenite Solution Used in Titration of 2-ml Aliquots	Moles of Periodate Consumed per Mole of Substance
5	3.15	0.91
10	3.14	0.97
20	3.13	1.04
60	3.10	1.23
180	3.09	1.29
24 hrs.	3.01	1.83

Table IV

Periodate Oxidation of N,N'-Diacetate of Compound R_{ds} 0.80 (XVI)

The amount of XVI-dihydrochloride used for the selective acetylation was 7.40 mg. The concentrations of the sodium metaperiodate solution and the sodium arsenite solution were 0.2461 M and 0.02977 M, respectively.

Time (min.)	Volume (ml) of Sodium Arsenite Solution Used in Titration of 2-ml Aliquots	Moles of Periodate Consumed per Mole of Substance
3	3.29	0.22
15	3.24	0.91
35	3.22	1.11
60	3.22	1.18
110	3.21	1.32
18 hrs.	3.14	2.27

Table V

Periodate Oxidation of N,N'-Diacetate of Compound R_{ds} 0.96 (XVII)

The amount of XVII-dihydrochloride used for the selective acetylation was 11.75 mg. The concentrations of the sodium metaperiodate solution and the sodium arsenite solution were 0.2495 M and 0.02977 M, respectively.

Time (min.)	Volume (ml) of Sodium Arsenite Solution Used in Titration of 2-ml Aliquots	Moles of Periodate Consumed per Mole of Substance
20	3.11	1.97
30	3.10	2.05
20 hrs.	3.10	2.05

Table VI

Periodate Oxidation of N,N'-Diacetate of Compound R_{ds} 1.15 (XVIII)

The amount of XVIII-dihydrochloride used for the selective acetylation was 10.96 mg. The concentrations of the sodium metaperiodate solution and the sodium arsenite solution were 0.2461 M and 0.02977 M, respectively.

Time (min.)	Volume (ml) of Sodium Arsenite Solution Used in Titration of 2-ml Aliquots	Moles of Periodate Consumed per Mole of Substance
5	3.295	0.10
31	3.280	0.21
20 hrs.	3.295	0.10

Table VII

Periodate Oxidation of N,N'-Diacetate of Compound R_{ds} 1.35 (XIX)

The amount of XIX-dihydrochloride used for the selective acetylation was 11.20 mg. The concentrations of the sodium metaperiodate solution and the sodium arsenite solution were 0.2461 M and 0.02977 M, respectively.

Time (min.)	Volume (ml) of Sodium Arsenite Solution Used in Titration of 2-ml Aliquots	Moles of Periodate Consumed per Mole of Substance
5	3.30	0.06
10	3.295	0.09
40	3.29	0.13
100	3.29	0.13
340	3.28	0.19
22 hrs.	3.28	0.19

Table VIII

Periodate Oxidation of N,N'-Diacetyl-2-deoxystreptamine (XIV)

The amount of 2-deoxystreptamine dihydrochloride used for the selective acetylation was 13.25 mg. The concentrations of the sodium metaperiodate solution and the sodium arsenite solution were 0.2480 M and 0.02977 M, respectively.

Time (min.)	Volume (ml) of Sodium Arsenite Solution Used in Titration of 2- ml Aliquots	Moles of Periodate Consumed per Mole of Substance
10	3.28	0.36
20	3.27	0.42
60	3.24	0.62
120	3.16	1.15
240	3.10	1.51
19 hrs.	3.00	2.21

Table IX

Periodate Oxidation of Methyl α-D-Glucopyranoside (XII)

A sample of 13.40 mg of methyl α-D-glucopyranoside (XII) was used. The concentrations of the sodium metaperiodate solution and the sodium arsenite solution were 0.2461 M and 0.02941 M, respectively.

Time (min.)	Volume (ml) of Sodium Arsenite Solution Used in Titration of 2-ml Aliquots	Moles of Periodate Consumed per Mole of Substance
3	3.32	0.14
8	3.28	0.35
20	3.22	0.67
35	3.16	1.00
50	3.14	1.10
65	3.10	1.31
80	3.06	1.53

Table X

Periodate Oxidation of Methyl α-D-Mannopyranoside (XIII)

A sample of 8.60 mg of methyl α-D-mannopyranoside (XIII) was used. The concentrations of the sodium metaperiodate solution and the sodium arsenite solution were 0.2451 M and 0.02941 M, respectively.

Time (min.)	Volume (ml) of Sodium Arsenite Solution Used in Titration of 2-ml Aliquots	Moles of Periodate Consumed per Mole of Substance
6	3.26	0.61
12	3.22	0.94
20	3.18	1.28
30	3.16	1.44
50	3.15	1.52

Part II. Attempted Stereospecific Synthesis of 2-Deoxystreptamine

Preparation of Methyl 2,3,4-Tri-O-methyl-D-xylopyranosides (XXXIII)

A crude anomeric mixture of methyl 2,3,4-tri-O-methyl-D-xylopyranosides (XXXIII) was prepared from D-xylose according to the method employed in the preparation of methyl tetra-O-methyl-D-glucopyranosides (132). From 27 g (0.18 mole) of D-xylose, 30.3 g of a lightly yellowish syrup was obtained. This syrup was subjected to a further methylation using methyl iodide in dimethylformamide (133) to ensure completeness of the methylation. Although this additional step caused a reduction in yield, it gave a colorless product (60 g from 90.6 g of crude product) that was free from hydroxyl groups and that crystallized on standing. The final yield was 53.9%.

Infrared data (in $CHCl_3$, cm^{-1}).- Methoxy, 1100 (strong). Others: 3000, 2940, 2920, 2840, 1460, 1445, 1385, 1370 and 1155. No hydroxyl.

Dimethyl 2,3,4-Tri-O-methyl-xylarate (XXXII)

To 77 ml (1.2 moles) of 70% nitric acid at 60° was added 0.1 g of sodium nitrite (131). An anomeric mixture of methyl 2,3,4-tri-O-methyl-D-xylopyranosides (41 g, 0.2 mole) was introduced in portions over a period of 20 minutes while the reaction mixture was stirred magnetically. The temperature

of the mixture was maintained at 55-60° by external cooling with cold water and the reaction was allowed to continue for one hour. The oxidation mixture was then cooled in an ice-water bath and partially neutralized by adding 40% aqueous potassium hydroxide solution until the pH was about 1.0. To this mixture was added 50 ml of methanol, and the potassium nitrate thereby deposited was filtered off. The filtrate was concentrated to a syrup and another 100 ml of absolute methanol was added. Inorganic salt was filtered off, and the filtrate was evaporated to give the dicarboxylic acid as a yellow glass (yield, 45.4 g). For esterification, 42 g of the crude acid was boiled under reflux for six hours in 300 ml of absolute methanol containing 7 g of hydrogen chloride. Upon cooling, sodium bicarbonate was added to pH 5. The filtered solution was concentrated to a syrup which was distilled under reduced pressure. The fraction (22.9 g) which distilled over at 138-141°/11.5 mm (reported (130), 132°/12 mm) was collected. The yield was 49.7%. Other boiling points: 115-118°/3 mm, 101-104.5°/0.42 mm. n_D^{28}, 1.4394 (reported (130), n_D^{15}, 1.4402). The n.m.r. spectrum (Fig. 17) was taken in $CDCl_3$.

Anal. Calcd. for $C_{10}H_{18}O_7$ (250.2): C, 48.01; H, 7.25; OCH_3, 62.03. Found: C, 48.93; H, 7.44; OCH_3, 61.57.

Infrared bands of the syrup, (in cm^{-1}): Carbonyl, 1740 (strong). Ester, 1195 (strong). Ether (CH_3-O-C), 1100 (strong) No hydroxyl. Others; 3000,2950, 2840, 1440, 1360, 1265, 1035, 1015, 990 and 850.

2,3,4-Tri-O-methyl-xylaric amide was prepared according to Hirst and Purves (130). From 5.7 g of the ester XXXII was obtained 1.6 g of crystals which on recrystallization from methanol melted at 193-194°(reported (130), 194-195°). The yield was 31.9%.

Infrared bands (in cm^{-1}).- Amide ($-NH_2$), 3450 (strong), 3200 (strong). Amide I, 1670 (strong). Amide II, 1600 (strong). Ether ($C-O-CH_3$), 1100 (strong, broad). No carbomethoxyl. Others; 1190, 1140, 1040, 920, 865, 820 and 720.

Attempted Cyclization of Glutaronitrile with Methylene Dimagnesium Diiodide (XXXV)

Methylene dimagnesium diiodide (XXXV) was prepared according to Fidler et al. (138). Magnesium (5 g) was covered with 30 ml of absolute ether and the mixture was warmed to boiling. A mixture of methylene iodide (28 g) and absolute ether (100 ml) was dropped in slowly with mechanical stirring. The reaction mixture separated into two layers as the reaction went on. After all the methylene iodide had been added the upper layer of the reaction mixture was removed by means of a suction tube. The lower layer which consisted of XXXV was separated from unreacted magnesium (about 1.5 g) by decantation. (a) A mixture of glutaronitrile (5.6 g) and absolute ether (150 ml) was added dropwise to the Grignard reagent XXXV. The mixture was stirred at reflux temperature for 20 hours. Upon

cooling to -20° the mixture was decomposed with 60 g of ammonium chloride and 500 g of crushed ice. The ether layer was separated and the aqueous layer was extracted three times with 70 ml portions of ether. The combined ether portions were dried over sodium sulfate and evaporated to give 3.15 g of unreacted glutaronitrile that was identified by its infrared spectrum. An aliquot of the aqueous layer left after extraction with ether was evaporated to a residue that was triturated with ether. An ether-insoluble, solid part was separated; its infrared spectrum showed no band at 1540-1640 cm^{-1} which would have been characteristic of 1,3-cyclohexanedione. Additional unreacted glutaronitrile was recovered from the ether-soluble portion.

(b) To methylene dimagnesium diiodide (XXXV) prepared from 5 g of magnesium and 28 g of methylene iodide was added a mixture of glutaronitrile (2.8 g) and tetrahydrofuran (200 ml). The mixture was heated to boiling and solvent was distilled off until the temperature reached 60°. More tetrahydrofuran (50 ml) was added and the mixture was refluxed and stirred overnight. The reaction mixture was decomposed as described above. Neither ether extract part nor aqueous part contained any 1,3-cyclohexanediimine or 1,3-cyclohexanedione as judged from infrared spectra and ferric chloride tests.

Attempted Cyclization of 2,3,4-Tri-O-methyl-Xylaryl Chloride (XXXVI) with Methylene Dimagnesium Diiodide (XXXV)

2,3,4-Tri-O-methyl-xylaric acid (XXXVII) was prepared according to Hirst and Purves (130). To 10.4 g of the diester XXXII was added a mixture of 12 g of barium hydroxide and 250 ml of water. The mixture was heated at 85° for one hour. Removal of the barium ions was done with Rexyn RG50(H^+) cation exchanger, rather than with sulfuric acid as described (130). The filtrate was evaporated to yield 7.8 g (84%) of glassy diacid XXXVII which showed the following infrared bands (syrup, in cm^{-1}). Carboxyl, 3450, 2600, 1720. Ether, 1090 (strong). Others; 2950, 2850, 1440, 1190, 1020 and 860. Hirst and Purves (130) considered the diacid so prepared as practically pure.

To 1.1 g of the diacid XXXVII was added 3.6 ml of thionyl chloride and the mixture was heated at 40-50° for 3 hours (145). The excess thionyl chloride was removed by evaporation, and 1.5 g of 2,3,4-tri-O- methyl-xylaryl chloride (XXXVI) was obtained. Silver nitrate solution gave positive test.

Infrared data (syrup; in cm^{-1}).- Acyl chloride, 1780 (broad). Ether 1100 (broad). No hydroxyl. Others; 2950, 2820, 1450, 1350, 1180, 1030, 990, 940, 920, 870, 825, 785, 740 and 710.

To methylene dimagnesium diiodide (XXXV) prepared from 4.3 g of magnesium was added a mixture of 6.6 g of the diacyl

chloride XXXVI amd 80 ml of ether. A brownish red solid was formed immediately. The mixture was stirred for one hour at reflux temperature and then left overnight at room temperature. The ether solution was separated from the brownish red solid by decantation, washed with dilute sodium bicarbonate solution and then dried over anhydrous sodium sulfate. The filtrate on evaporation yielded a small amount of syrup which was not the diketone XXVII as shown by the infrared spectrum and a negative test with ferric chloride solution. It was also found that this syrup did not form a 2,4-dinitrophenylhydrazone.

The brownish red solid from which the ether solution had been decanted was decomposed with ice-water, and ether was added. The ether layer was separated. The aqueous layer was extracted three times with ether. The combined ether extracts were dried over anhydrous sodium sulfate. Evaporation yielded a brownish syrup which consisted mainly of acid XXXVII as shown by its infrared spectrum. It was also found that this syrup did not form any crystalline oxime or 2,4-dinitrophenylhydrazone. The aqueous layer left after the extraction with ether was similarly checked and was also found to contain no diketone.

Methylene dimagnesium diiodide (6.5 g) prepared by the method described above was heated in an oil bath under argon gas at 140-150° for 5 hours according to Ziegler (140). A light yellowish solid was obtained. A solution of the diacyl chloride XXXVI (5.2 g) in benzene (50 ml) was added. The

mixture was stirred and refluxed for 2 hours. Cold dilute hydrochloric acid was added and the benzene layer was separated. The benzene layer was shaken with mercury to remove a pink color due to the presence of free iodine. Drying over anhydrous sodium sulfate and evaporation yielded a syrup which was found to consist of the anhydride XLII.

Infrared bands (in cm^{-1}; syrup).- Anhydride, 1785 (strong), 1740 (strong). Ether, 1120 (strong). Others; 2950, 2830, 1450, 1190 and 945.

The aqueous solution left after separation from the benzene layer was evaporated to dryness and an infrared spectrum of the residue was taken . No diketone XXVII was present.

Attempted Cyclization of 2,3,4-Tri-O-methyl-xylaryl Chloride (XXXVI) with Methylene Cadmium

To methylene dimagnesium diiodide (XXXV) obtained from 2.6 g of magnesium was added 18.3 g of anhydrous cadmium chloride (141b). The ether was distilled off and a paste-like residue was obtained. Benzene (150 ml) was added to the residue and a solution of the diacyl chloride (XXXVI)(6 g) and benzene (50 ml) was dropt in immediately. The mixture was stirred and heated at 77° for 20 minutes; it became purple during this period. The reaction mixture was decomposed with 400 g of 5% sulfuric acid in ice and extracted with 260 ml of benzene,

in three portions. The benzene extract was washed with water, 10% sodium bicarbonate solution, again with water, and was finally dried over anhydrous sodium sulfate. Evaporation yielded 0.68 g of a syrup which was shown by its infrared spectrum to consist of the anhydride XLII.

Infrared spectrum (syrup, in cm^{-1}).- Anhydride, 1790 (strong), 1745 (strong). Ether, 1120 (strong). Others; 2950, 2850, 1450, 1190 and 945.

The sodium bicarbonate washing was acidified and was evaporated to give a syrup along with inorganic salt. The syrup consisted of the diacid XXXVII as shown by the infrared spectrum.

The aqueous layer left after separation from the benzene layer was evaporated and the residue was checked in the infrared. No diketone XXVII was found.

Attempted Cyclization of Dimethyl 2,3,4-Tri-O-methyl-xylarate (XXXII) with Methyl Acetate

To a solution of 2.5 g (0.01 mole) of dimethyl 2,3,4-tri-O-methyl-xylarate (XXXII) in 20 ml of absolute ether was added 1 g of solid sodium methoxide (150) and 1.2 ml (0.015 mole) of methyl acetate. The mixture which became yellowish during the mixing was refluxed for two hours. The ether was distilled off to give a pasty, reddish residue which was subsequently heated on an oil-bath at 100° for 4.5 hours. Upon cooling, the residue was acidified with dilute sulfuric

acid, and the acidic solution was extracted five times with 30-ml portions of ether. The combined ether extracts were treated with charcoal and then dried over anhydrous sodium sulfate. Removal of the ether yielded 0.8 g of a syrup which was not the expected diketone XXXIX as shown by the infrared spectrum and a negative test towards ferric chloride solution. The aqueous layer left after the ether extraction was also checked by infrared spectroscopy. No diketone XXXIX was present.

Attempted Cyclization of Dimethyl 2,3,4-Tri-O-methyl-xylarate (XXXII) with Ethyl Acetate

To a mixture of 1.25 g (0.005 mole) of dimethyl 2,3,4-tri-O-methyl-xylarate (XXXII) and 0.5 ml (0.005 mole) of ethyl acetate was added 0.68 g (0.01 mole) of sodium ethoxide (150). The mixture which became brownish was heated at 100° for 30 minutes. After cooling, ethanol was added and sodium ion was removed with Dowex 50(H^+). The filtrate was evaporated to give a syrup which was not the expected diketone XXXIX as shown by the infrared spectrum and a negative test towards ferric chloride solution.

Preparation of Methyl Hydrogen 2,3,4-Tri-O-methyl-DL-xylarate (XLI)

(a) This method originated from Breslow et al.(144). To a mixture of 1.0 g (0.004 mole) of dimethyl 2,3,4-tri-O-methyl-

xylarate (XXXII) and 3 ml of absolute methanol was added a solution of 0.224 g (0.004 mole) of potassium hydroxide in 3 ml of absolute methanol. A yellowish precipitate was formed immediately. The mixture was stirred at room temperature for one hour. Water was added in a quantity just enough to dissolve the precipitate. Potassium ion was removed by Dowex 50(H^+). The filtrate was evaporated to give a yellowish syrup which was distilled under diminished pressure. The fraction (0.576 g) distilling over at 132°/3mm. was a glassy syrup. The yield was 61%. The n.m.r. spectrum (Fig. 18) was taken in deuterochloroform.

Anal. Calcd. for $C_9H_{16}O_7$(236.22): C, 45.76; H, 6.83; OCH_3, 53.29; COOH, 19.06. Found: C, 45.96; H, 6.95; OCH_3, 52.55; COOH, 18.45.

Infrared data (in cm^{-1}; syrup).- Carboxyl, 3500, 3200 (strong, broad), 2600 (medium, broad), 1730 (strong, broad). Ester, 1190 (strong). Methoxy, 1100 (strong). Others; 1440, 1340, 1260, 1020, 900, 850 and 650.

(b) A mixture of 6 g (0.027 mole) of 2,3,4-tri-O-methyl-xylaric acid (XXXVII) and 11 ml of acetic anhydride was heated in a water bath at 70° for 3 hours (151). Acetic acid and excess acetic anhydride were removed at 60° in vacuo. To the residue was added 1 ml of absolute methanol and the mixture was heated at 60° for one hour (145). The excess methanol was evaporated and the residue was distilled under diminished pressure. The fraction (3.15 g) distilling over at 117-136°/0.175 mm. was

collected. (Yield, 49.3%). The infrared and n.m.r. spectra of this product were identical with those of the product obtained in method (a).

Preparation of γ-Carbomethoxy-D,L-xylo-trimethoxybutyryl Chloride (XLIII)

(a) This method originated from Adams and Ulich (146). A mixture of 1.3 g (0.0055 mole) of methyl hydrogen 2,3,4-tri-O-methyl-D,L-xylarate (XLI), 5 ml of oxalyl chloride and 30 ml of benzene was warmed to start the reaction. After the evolution of gases had ceased the mixture was boiled for 2 hours. Excess oxalyl chloride and benzene were distilled off and the residue was distilled under diminished pressure. The fraction (0.754 g) which distilled over at 104-107°/0.40 mm. was a colorless syrup. The yield was 55%. The n.m.r. spectrum was taken in deuterochloroform (Fig. 19).

Anal. Calcd. for $C_9H_{15}O_6Cl$ (254.67): C, 42.44; H, 5.94; Cl, 13.92; OCH_3, 48.74. Found: C, 42.21; H, 6.12; Cl, 12.86 ; OCH_3, 49.24.

13.76

Infrared data (syrup; in cm^{-1}).- Acyl chloride C = O, 1800 (strong). Carbomethoxy C = O, 1740 (strong). Carbomethoxy-$\underset{\underset{O}{\|}}{C}$-O-$CH_3$, 1190 (strong). Methoxy group, 1100 (strong). No hydroxyl. Others; 2990, 2940, 2835, 1435, 1345, 1270, 1010, 945, 860 and 770.

(b) A mixture of 1.18 g (0.005 mole) of methyl hydrogen 2,3,4-tri-O-methyl-D,L-xylarate (XLI) and 0.72 ml (0.01 mole) of thionyl chloride was heated at 30-40° for 3 hours (145). The excess thionyl chloride was distilled off and the residue was distilled under diminished pressure. The fraction (0.73 g) which distilled over at 120-123°/15 mm. was a colorless syrup. The yield was 57.2%. The n.m.r. spectrum was taken in deuterochloroform. It was found that the infrared and n.m.r. spectra of this product were identical with those of the product obtained in method (a). Other boiling points found for the acyl chloride XLIII were; 108-111°/0.50 mm. and 110-114°/1.60 mm.

Preparation of Methyl 6-Diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV)

Diazomethane was prepared from 6 g of nitrosomethylurea, 17.5 ml of 50% potassium hydroxide solution and 70 ml of ether (152). The distilled diazomethane in ether was dried over pellets of potassium hydroxide for three hours before use. To the magnetically stirred diazomethane solution was added a solution of 2.7 g (0.0106 mole) of γ-carbomethoxy-D,L-xylo-trimethoxy-butyryl chloride (XLIII) in ether while the reaction flask was cooled externally with ice-water. A spontaneous evolution of gas was observed. After all the acyl chloride had been added the mixture was stirred at room temperature for 2 hours. The ether and excess diazomethane were removed by

evaporation under diminished pressure. A yellowish syrup (2.46 g) was obtained (yield, 89.2%). The n.m.r. spectrum (Fig. 20) was taken in deuterochloroform.

Infrared data (in cm^{-1}; syrup).- C-H in $-\underset{H}{C}=\overset{+}{N}=\overset{-}{N}$, 3100 (weak). Diazo bond in $-\underset{H}{\overset{+}{C}}-\overset{-}{N}\equiv N$ (or $-\underset{H}{C}=N\equiv N$), 2110 (strong), in $-\underset{H}{C}=\overset{+}{N}=\overset{-}{N}$, 1630 (strong). Carbonyl, 1740 (strong). Carbomethoxy ($-\overset{O}{\overset{\|}{C}}-O-CH_3$), 1195 (strong). Methoxy ($C-O-CH_3$), 1105 (strong). Others; 2995, 2940, 2840, 1450, 1345, 1270, 1040 and 1010.

Reaction between Methyl 6-Diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) and Hydriodic Acid

To a chloroform solution (15 ml, ethanol-free) of methyl 6-diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) (1.4 g), contained in a separatory funnel, was added 7 ml of 47% hydriodic acid (148). The mixture was shaken carefully. A copious evolution of nitrogen and formation of iodine were observed; the heat of reaction was considerable. Shaking was continued until there was no further evolution of nitrogen (a few minutes). The mixture was diluted with water and the chloroform layer was separated and washed with water, dilute aqueous sodium thiosulfate and again with water. The chloroform solution was then dried over anhydrous sodium sulfate and on evaporation yielded

a brownish syrup (1.24 g) which was distilled under diminished pressure. The fraction (0.652 g) which distilled over at 117-119°/3 mm. was a colorless syrup. This syrup gave a positive color test (deep violet) with methanolic ferric chloride solution.

Anal. Found: C, 46.82; H, 6.71. This analysis fits best an empirical formula $C_{12}H_{20}O_9$ (308.28), for which is calculated C, 46.75; H, 6.54, whereas for the expected compound XL ($C_{10}H_{18}O_6$) the values required are C, 51.27; H, 7.75. The n.m.r. spectrum of the product in deuterochloroform is shown in Fig. 21.

Infrared data (in cm^{-1}; syrup).- Carbonyl, 1745 (strong). Carbomethoxy, 1195 (strong). Methoxy, 1110 (strong). No hydroxyl. Others; 2995, 2940, 2840, 1640, 1435, 1350, 1270 and 1000.

A 2,4-dinitrophenylhydrazone of the product was obtained as brick-red crystals by heating with 2,4-dinitrophenylhydrazine in ethanol for 15 minutes. These crystals turned violet in alcoholic potassium hydroxide. On heating the 2,4-dinitrophenylhydrazone decomposed over a wide range 100-140°. The sample for analysis was dried at 56° <u>in vacuo</u> for several hours.

Anal. Found: C, 44.36; H, 4.16; N, 18.85; OCH_3, 12.61. The empirical formula calculated from this analysis was $C_{22}H_{24}N_8O_{12}$.

Infrared data (in cm^{-1}).- Carbonyl, 1740 (medium). 1,2,4-Tri-substituted Phenyl, 880 (weak), 830 (medium).

Others; 1610 (strong), 1585 (strong), 1490 (strong), 1330, 1310, 1275, 1220, 1135, 915, 740 and 715.

Catalytic Hydrogenation of Methyl 6-Diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) with Palladium on Charcoal

A suspension of 0.1 g of 10% palladium on charcoal and 25 ml of ethyl acetate was prehydrogenated. Methyl 6-diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV) (0.2 g) was then introduced and the hydrogenation was carried out at room temperature and ordinary pressure. The reaction was followed by the infrared spectrum of the product, and it was found that the two strong peaks at 2110 and 1630 cm^{-1}, as well as the band at 3100 cm^{-1} disappeared. A yellow syrup was obtained by evaporating the hydrogenated solution. The n.m.r. spectrum in deuterochloroform is shown in Fig. 22.

Infrared data (in cm^{-1}; syrup).- Carbonyl, 1740 (strong). Carbomethoxy, 1195 (strong). Methoxy, 1105 (strong). Others; 2995, 2940, 2840, 1440, 1350, 1260 and 1040.

Reaction between γ-Carbomethoxy-D,L-xylo-trimethoxybutyryl Chloride (XLIII) and Dimethyl Cadmium

Methyl magnesium bromide was prepared from 0.70 g of magnesium and 2.5 ml of methyl bromide in 30 ml of ether. Dimethyl cadmium was then prepared from this Grignard reagent by adding anhydrous cadmium chloride (4.0 g) (141b). To the

mixture of dimethyl cadmium and benzene was added a solution of 3.4 g of γ-carbomethoxy-D,L-xylo-trimethoxybutyryl chloride (XLIII) in 15 ml of benzene. After the addition of the acyl chloride was complete the mixture was stirred and heated under reflux for an hour. The reaction mixture was cooled in an ice bath and decomposed by careful addition of 30 g of ice-water followed by sufficient 20% sulfuric acid to give two clear phases. The aqueous layer was separated and extracted with two 30-ml portions of benzene. The benzene layer and extracts were combined and washed successively with water, 5% sodium bicarbonate, water and saturated sodium chloride solution. The benzene layer was finally dried over anhydrous sodium sulfate and gave, on evaporation, 2.5 g of a yellowish syrup. This syrup was distilled under diminished pressure. The fraction (0.69 g) which distilled over at 80-84°/0.30 mm. was a colorless liquid. The residue left after distillation was a brownish resin. The n.m.r. spectrum of the liquid in deuterochloroform is shown in Fig. 23.

Anal. Calcd. for $C_{10}H_{20}O_6$ (236.26): C, 50.81 ; H, 8.53. Found: C, 50.46 ; H, 8.40.

Infrared data (liquid, in cm^{-1}).- Ester-carbonyl, 1745 (strong). Carbomethoxy, 1190 (strong). Methoxy, 1100 (strong). Others; 2980, 2940, 2835, 1450, 1350 and 1260. No hydroxyl between 3100-3635.

CLAIMS TO ORIGINAL RESEARCH

1. Catalytic hydrogenation of 4,6-dinitropyrogallol afforded five new aminocyclitols that were isolated by column chromatography and characterized as crystalline hydrochlorides.
2. Periodate oxidation and proton magnetic resonance studies led to the following structural and configurational assignments for the new aminocyclitols:
   (a) 1α,3α-Diamino-4α,5α,6α-trihydroxycyclohexane (XV)
   (b) D,L-1α,3α-Diamino-4α,5α,6β-trihydroxycyclohexane (XVI)
   (c) D,L-1α,3β-Diamino-4α,5α,6α-trihydroxycyclohexane (XVII)
   (d) 1α,3α-Diamino-4α,6α-dihydroxycyclohexane (XVIII)
   (e) D,L-1α,3α-Diamino-4α,6β-dihydroxycyclohexane (XIX)
3. The following derivatives of the aminocyclitols were prepared in the course of these investigations.
   (a) Dipicrate of XV
   (b) Pentaacetate of XV
   (c) Pentaacetate of XVI
   (d) N,N'-Diacetate of XVII
   (e) Pentaacetate of XVII
   (f) N,N'-Diacetate of XVIII
   (g) Tetraacetate of XVIII
   (h) Tetraacetate of XIX

4. In the course of attempting a synthesis of 2-deoxystreptamine the following new compounds were obtained:

   (a) Methyl hydrogen 2,3,4-tri-O-methyl-D,L-xylarate (XLI)

   (b) γ-Carbomethoxy-D,L-xylo-trimethoxy-butyryl chloride (XLIII)

   (c) Methyl 6-diazo-6-deoxy-2,3,4-tri-O-methyl-D,L-xylo-5-hexulosonate (XLIV)

   (d) Compound $C_{12}H_{20}O_9$ from XLIV and hydriodic acid

   (e) Compound $C_{10}H_{20}O_6$ from XLIII and dimethyl cadmium

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