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The synthesis of methyl-2-acetamido-3,4,6-0-triacetyl alpha-D-talosaminide

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THE SYNTHESIS OF

METHYL-2-ACETAMIDO-3,4,6-O-TRIACETYL ALPHA-D-TALOSAMINIDE

A Thesis

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by

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CHAPTER I

INTRODUCTION

Carbohydrates in which one or more OH groups are replaced by an amino function are commonly designated as aminosugars. Chitin which constitutes an important skeletal substance of numerous invertebrate species has been the source of the most plentiful aminosugar, "Chitosamine" (2-amino 2-deoxy D-glucose) which was first isolated by Ledderhose (1) in 1878. As determined by Fischer (4), Cutler (5), and Cox (8) it was found to have the D-glucose configuration.

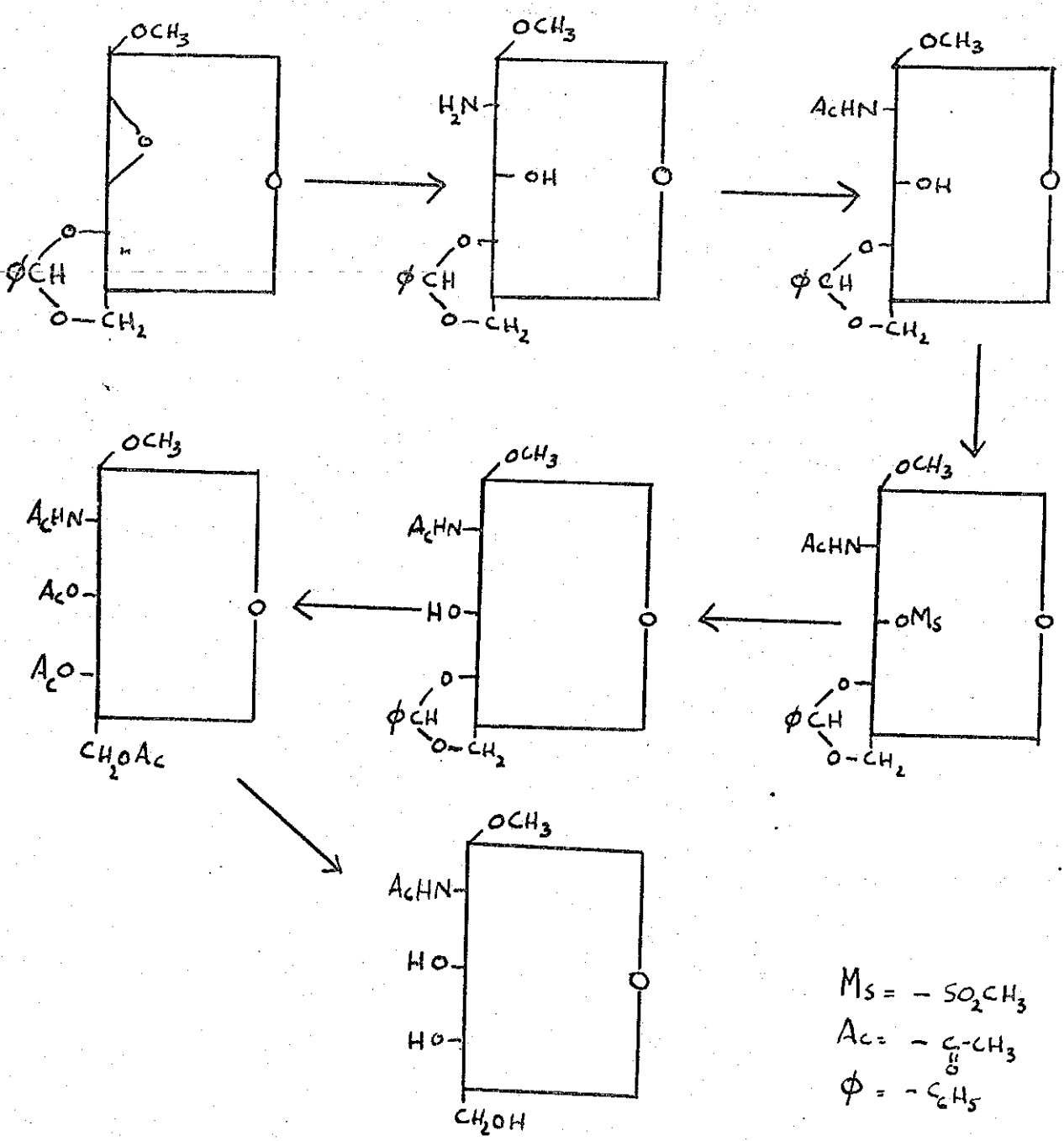
After the initial discovery of aminosugars there was a period of about forty years in which not much work was done in this new field. It was the presence of galactosamine found in mammalian tissues, invertebrates and higher plants (6) that created the considerable present interest. Aminosugars have been found in a variety of physiologically important compounds including structural polysaccharides, glycolipids, mucoproteins, and nucleotides (7). The presence of aminosugars has been confirmed in bacterial and fungal cell walls. Up to now, thirteen different aminosugars have been identified as components of bacteria or of their products the most common being glucosamine and galactosamine (6).

Amongst the very many different kinds of aminosugars, the ones that have aroused the most interest are the 2-amino 2-deoxy hexoses which are found in such well known antibiotics as streptomycin and neomycine (2). There are so far about forty-three antibiotics which are known to contain different aminosugars (6).

Aminosugars exhibit the same properties as other reducing aldohexoses, e. g. reduction of silver and cupric salts, oxidation to hexonic acids, reduction to alcohols and formation of glycosides.

The object of this project is to synthesize methyl N-acetyl α-D-talosaminide which has previously been prepared by Jeanloz (3) following a different route. The path that Jeanloz, Jeanloz, and Glazer (3) followed involved the preparation of methyl 2-acetamido 4, 6-benzylidene 2-deoxy α-D-idospyranoside by ammonolysis of methyl 2,3-anhydro 4,6-benzylidene α-D-guloside in a sealed tube and followed by N-acetylation. Methyl 2-acetamido 4,6-benzylidene 2-deoxy α-D-idospyranoside was treated with methane sulphonyl chloride to yield methyl 2-acetamido 4,6-benzylidene 3-O-mesyl α-D-idospyranoside which was converted to methyl 2-acetamido 4,6-benzylidene talopyranoside by treatment with sodium acetate in aqueous 2-methoxyethanol. This compound was debenzylidenated with aqueous acetic acid and acetylated to obtain methyl 2-acetamido 3,4,6-tri-O-acetyl talosaminide.

In short, the above route involved essentially a direct conversion from the idose series into the talose series which is illustrated in Scheme I.



Preparation of Methyl-2-Acetamido-2-deoxy-α-D-Talopyranoside
(Conversion of idose series into talose series)

Scheme I

The reaction which attracted the most interest in the above route is the conversion of the 3-O-mesyl derivative of the idospyranoside to the 2-acetamido derivative of the talopyranoside by treatment with sodium acetate in aqueous 2-methoxyethanol. Baker and Schaub (10) demonstrated for the first time in carbohydrate chemistry that sodium acetate in aqueous Methyl Cellosolve (2-methoxyethanol) causes elimination of one and two mesyl groups with configurational inversion. Although this is the first known example of this reaction in the carbohydrate field, the reaction has been described with trans-acetamino cyclohexanol-2-tosylate by McCasland, Clark and Carter (11). Quite generally this reaction has the limitation that the acetamino and the methanesulphonate groups must be in trans-position to each other. Only then can the methanesulphonate group be eliminated to result in the cis configuration. The inversion from a cis-to a trans- configuration was never demonstrated. This concept of neighbouring group participation has been put to use extensively by Jeanloz (12) in the aminohexoses series. In another variation of this concept Gross, Brendel and Zimmerman (13) carried out the epimerization of 2-benzamido-2-deoxy-D-glucose derivatives in an acidic medium, the result being that here the inversion of C-3 occurred with acyl migration to the oxygen.

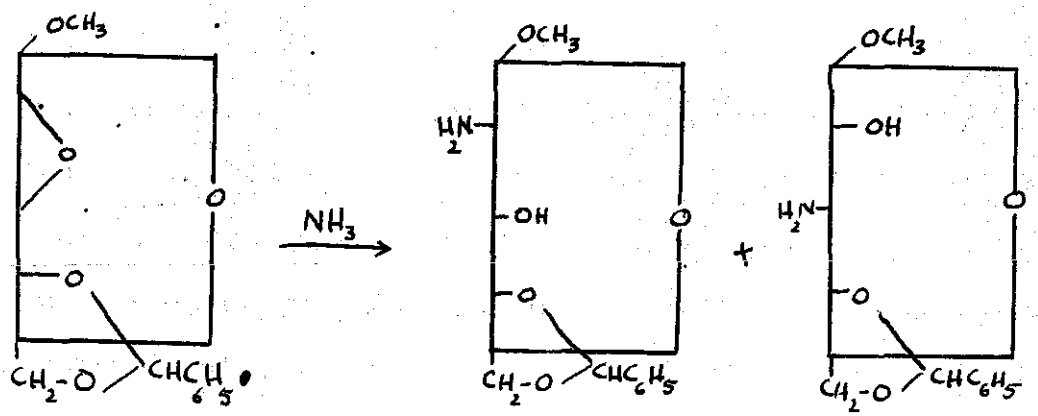
CHAPTER II

DISCUSSION

It is the purpose of this investigation to use the plentiful, inexpensive methyl∝-D-glucopyranoside for a convenient route to derivatives of talosamine.

Methyl∝-D-glucopyranoside I, which was bought in bulk from the Corn Products Refining Co., was treated with benzaldehyde / zinc chloride to yield methyl 4,6-benzylidene ∝-D-glucopyranoside II, (14) which was treated with methanesulphonyl chloride to yield methyl 4,6-benzylidene 2, 3-0- di methanesulphonyl ∝-D-glucopyranoside, III. The methanesulphonyl derivative was dissolved in dichloroethane and sodium methoxide to obtain methyl 2,3 anhydro-4,6-benzylidene ∝-D-allopyranoside, IV, by the method described by Richtmyer (15) for the preparation of an anhydro sugar from methyl 4,6-0-benzylidene 2,3-di-0-tolylsulphonyl ∝-D-glucopyranoside. The anhydro sugar was treated with aqueous ammonia in a sealed tube to obtain methyl 2-amino 4,6-0-benzylidene-∝-D-altropyranoside, V, as described by Meyers and Robertson (16).

The epoxy ring at the C-2 and C-3 position of the anhydro sugar opens to give a product with trans configuration because the nucleophile, NH_3, must attack from the back side. It seems as if the entering NH_3 group could attack both at C-2 and C-3 positions, resulting in a mixture of two products:



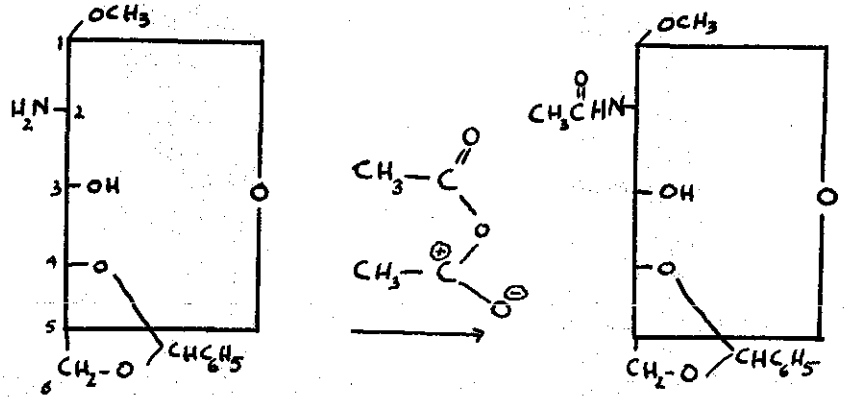
IV V (a) V (b)

Experimentally only V (a) is formed, resulting from the trans diaxial opening of the ring with a fixed conformation of the pyranoside ring due to the presence of the benzylidene bridge. It turns out that this behaviour follows a general rule that trans diaxial opening is favoured over trans equatorial opening (21), (22).

The 2-amino sugar, V, was treated with benzoic anhydride to yield methyl 2-benzamido 4,6-O-benzylidene 2-deoxy-∝-D-altropyranoside, VI, by the method described by Buss, Hough and Richardson (17). The benzamido derivative, VI, was methanesulphonated by the standard procedure to obtain methyl 2-benzamido 4,6-O-benzylidene 2-deoxy 3-O-methanesulphonyl-∝-D-altropyranoside VII. The proposed route called for the preparation of methyl 2-benzamido 4,6-O-benzylidene-∝-D-mannopyranoside, IX. We tried to obtain this compound through direct inversion of VII by treatment with

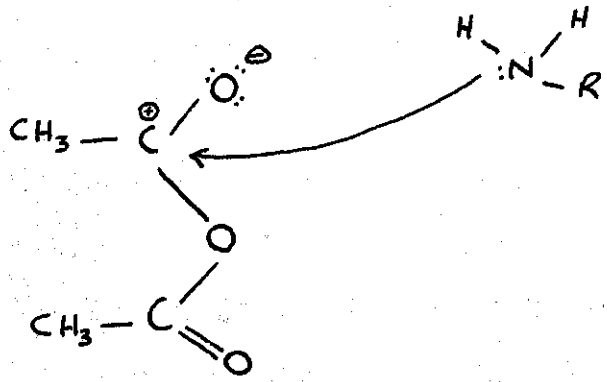
potassium acetate in aqueous 2-methoxyethanol. However, it turned out, that an oxazoline, 4',6'-benzylidene 2-phenyl-1'-O-methyl-∝-D-mannopyrano [2.'3:'4.5] Δ^2-oxazoline VIII, was formed instead. Indeed the formation of oxazolinium ions is proposed as an intermediate step in the conversion of trans-acetamino-2-methanesulphonate to cis-acetamino alcohol by treatment with sodium acetate / 2-methoxyethanol in anology to acetoxonium ions proposed by Winstein and Buckles (19). The identity of the oxazoline VIII to a compound previously prepared by Reckendorff (18) was shown by comparison to a sample which was prepared according to the published procedure (18). Repeated attempts to hydrolyse the oxazoline with alkali under different conditions failed to split the oxazoline group. Only a very minor fraction was cleaved. Generally, oxazolines with a phenyl group have proved to be very stable towards alkali. The reason may be seen in the stabilization of the oxazoline double bond by conjugation with the benzene ring. It was decided, therefore, to obtain 2-acetamido derivatives of talosamine instead of the benzamido derivatives. So compound V was treated with acetic anhydride in ethanolic solution to yield methyl 2-acetamido 4,6-O-benzylidene 2-deoxy-∝-D-altropyranoside X.

The mechanism of the selective N-acetylation with acetic anhydride is most probably the following:



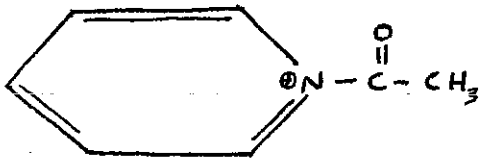
V X

Acylation is not possible at position C-4 and C-6 because the -OH groups at these positions are already protected by the benzylidene blocking group. The position where acylation can take place is either at the secondary -OH group at position C-3 or the primary $-NH_2$ group at position C-2. Since the basicity of the $-NH_2$ group is greater than that of the -OH group the acylating species,



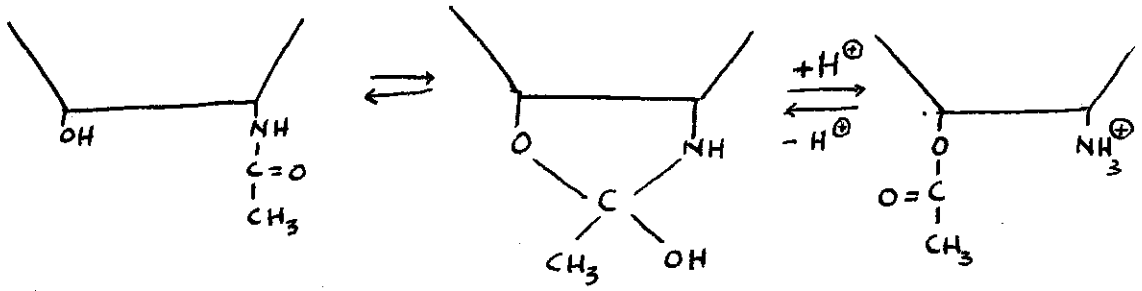
is attracted mainly to the nitro function. Further selectivity is achieved by using a solvent with a primary -OH group e. g., ethyl alcohol in excess. Pyridine is added to the reaction mixture only later, in order to liberate

unacetylated amino groups of any acetic acid formed; and a second acetylation is performed in the same way to bring the process to completion. If pyridine were added immediately the very active, unspecific species:



would be formed and acetylation would probably occur mainly on the -OH groups of the solvent, which is in large excess.

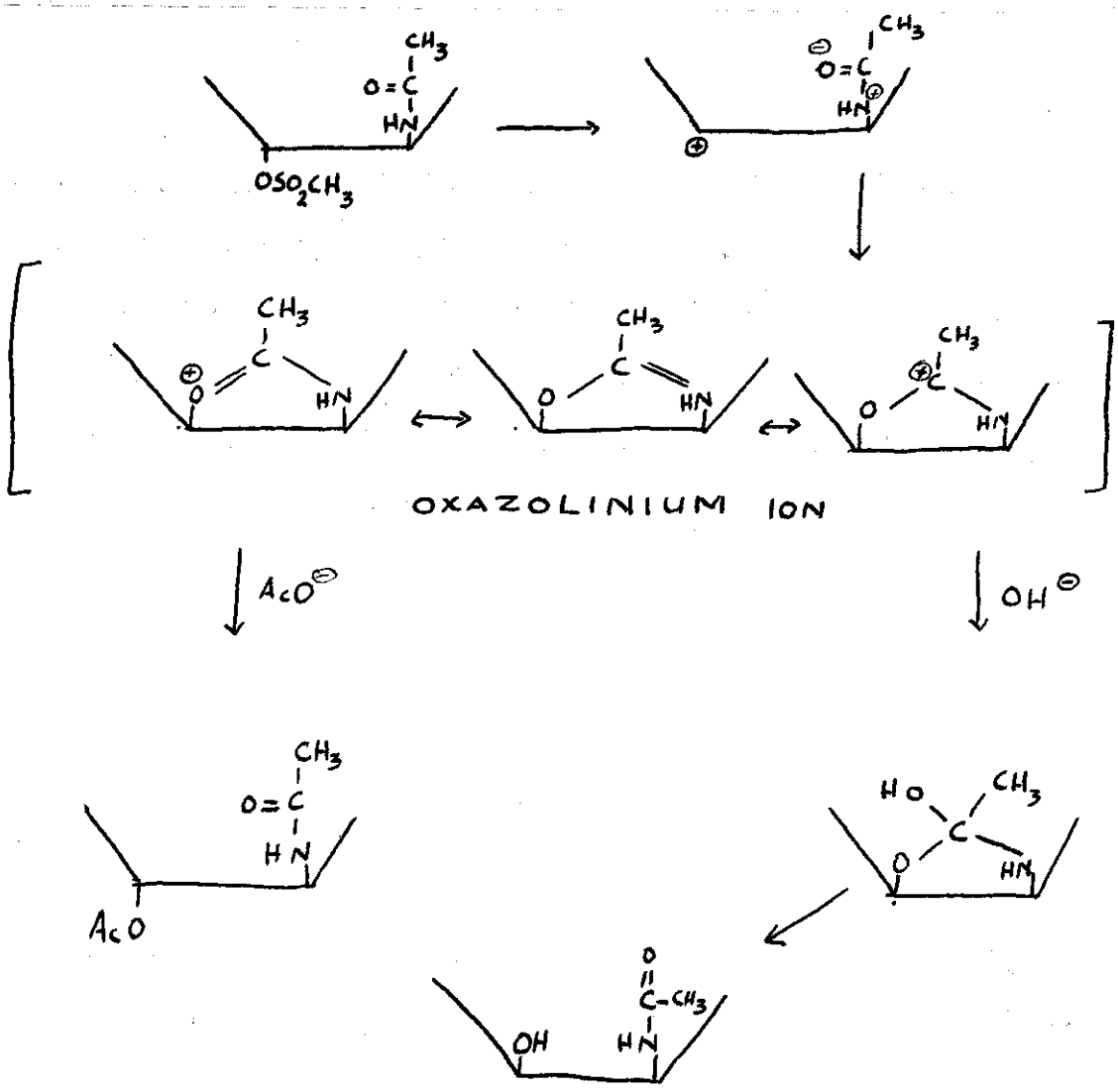
If acetylation were carried out in an acidic medium then O-acetylation would be preferred. These facts are easily correlated to the results of acyl migration under basic and acidic conditions respectively



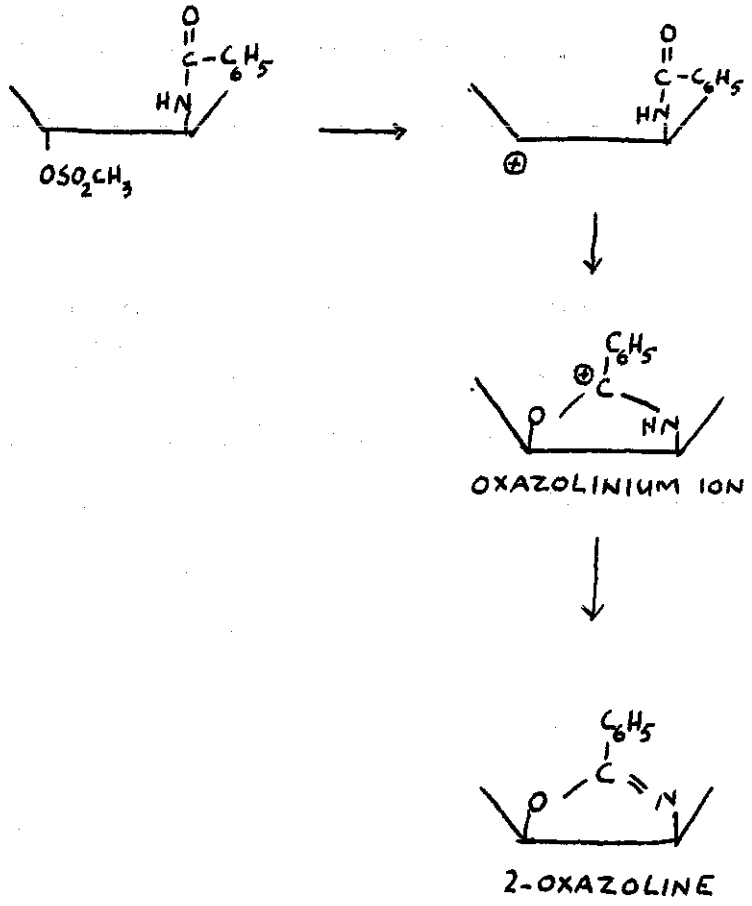
Compound X was treated with methanesulphonyl chloride to obtain methyl 2-acetamido 4,6-O-benzylidene 3-O-mesyl-α-D-altropyranoside XI, (17). This 3-O-mesyl derivative XI was subjected to treatment with aqueous methyl cellosolve and potassium acetate to yield methyl 2-acetamido 4,6-O-benzylidene-α-D-mannopyranoside, (XII), with inversion taking place at the C-3

position.

The inversion proceeds via the formation of an oxazolinium ion. The oxazolinium ion derived from the acetyl derivative, XI, is less stable than the one formed from the benzamido derivative, VII. This was the reason for choosing acetyl as the nitrogen protection group.

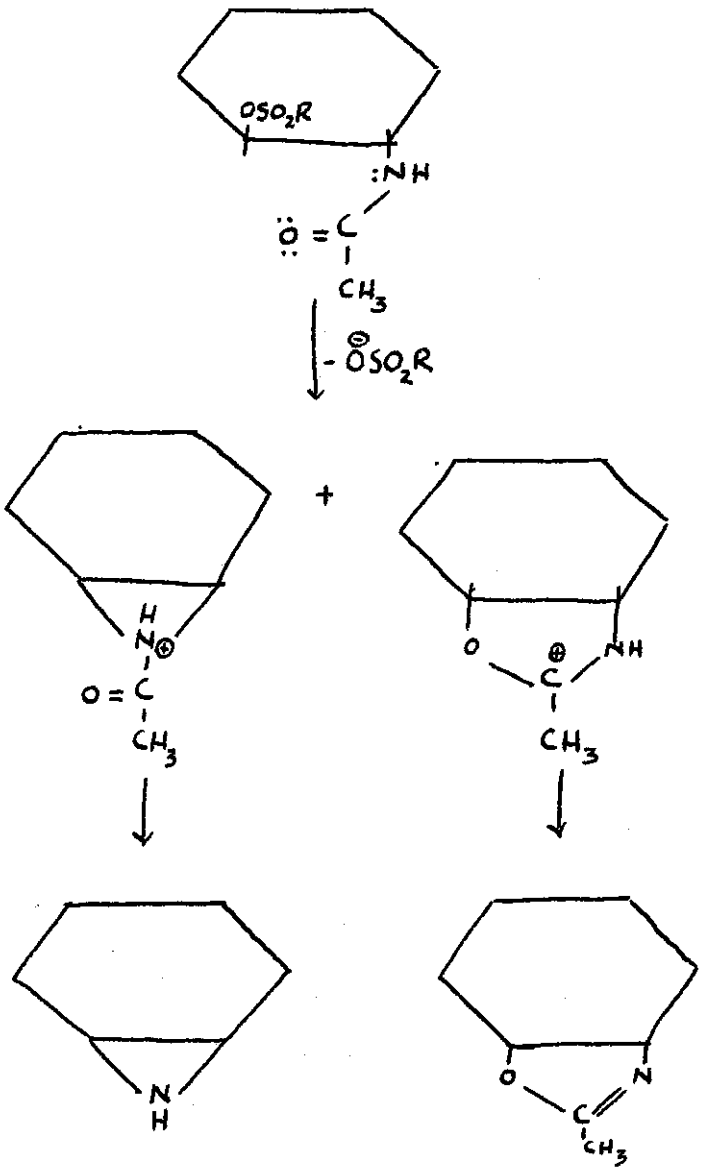


In the case of the benzamido derivative the oxazolinium ion is stabilized by losing a proton to form the 2-oxazoline, VIII, which was found very difficult to cleave.

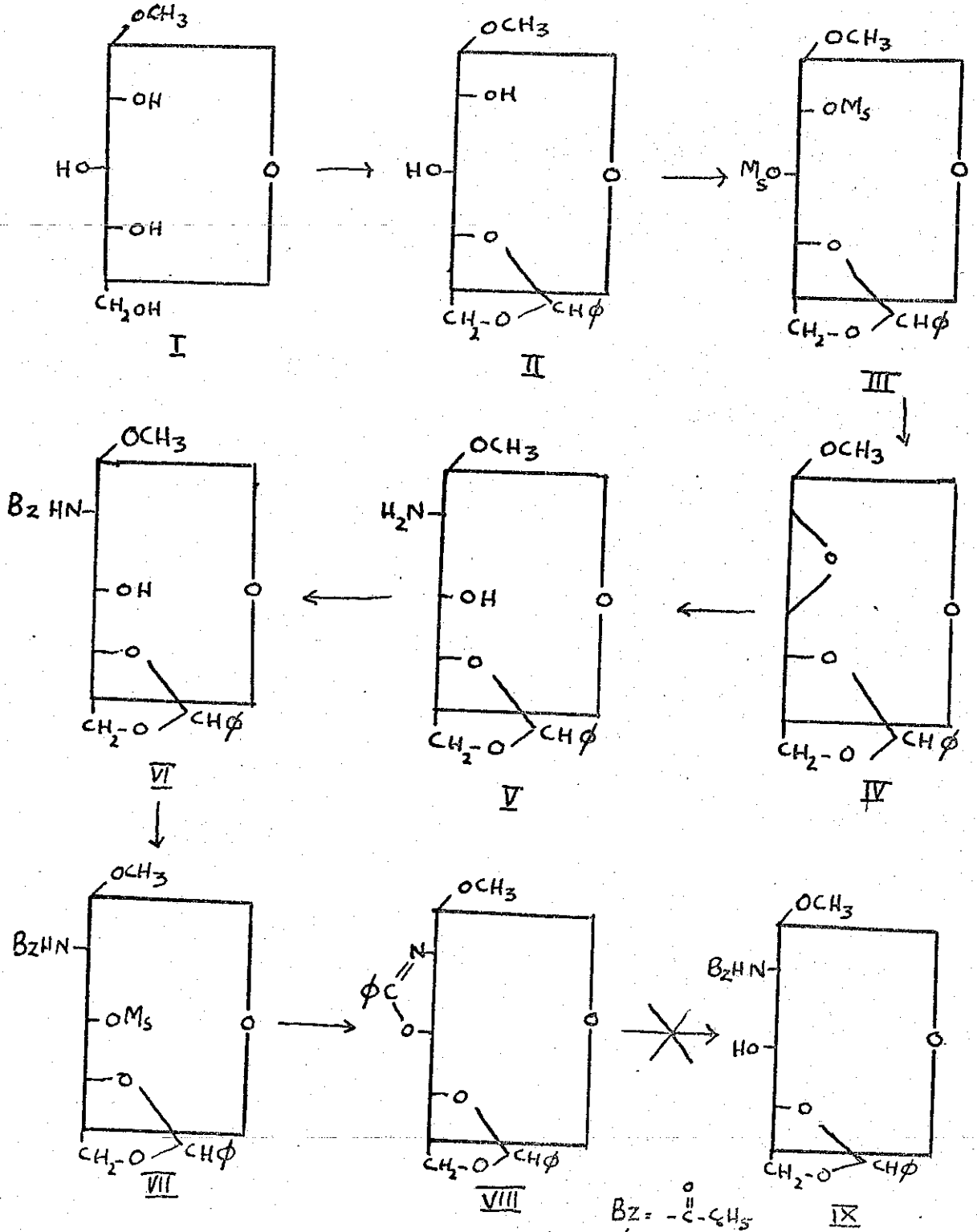


The reason why an aqueous medium was used, becomes apparent by consideration of the possible formation of (XVII) in an anhydrous medium. Here the oxazolinium ion would react, if at all, with a second inversion to give the already prepared altro derivative. Thus we would have net retention of configuration (19).

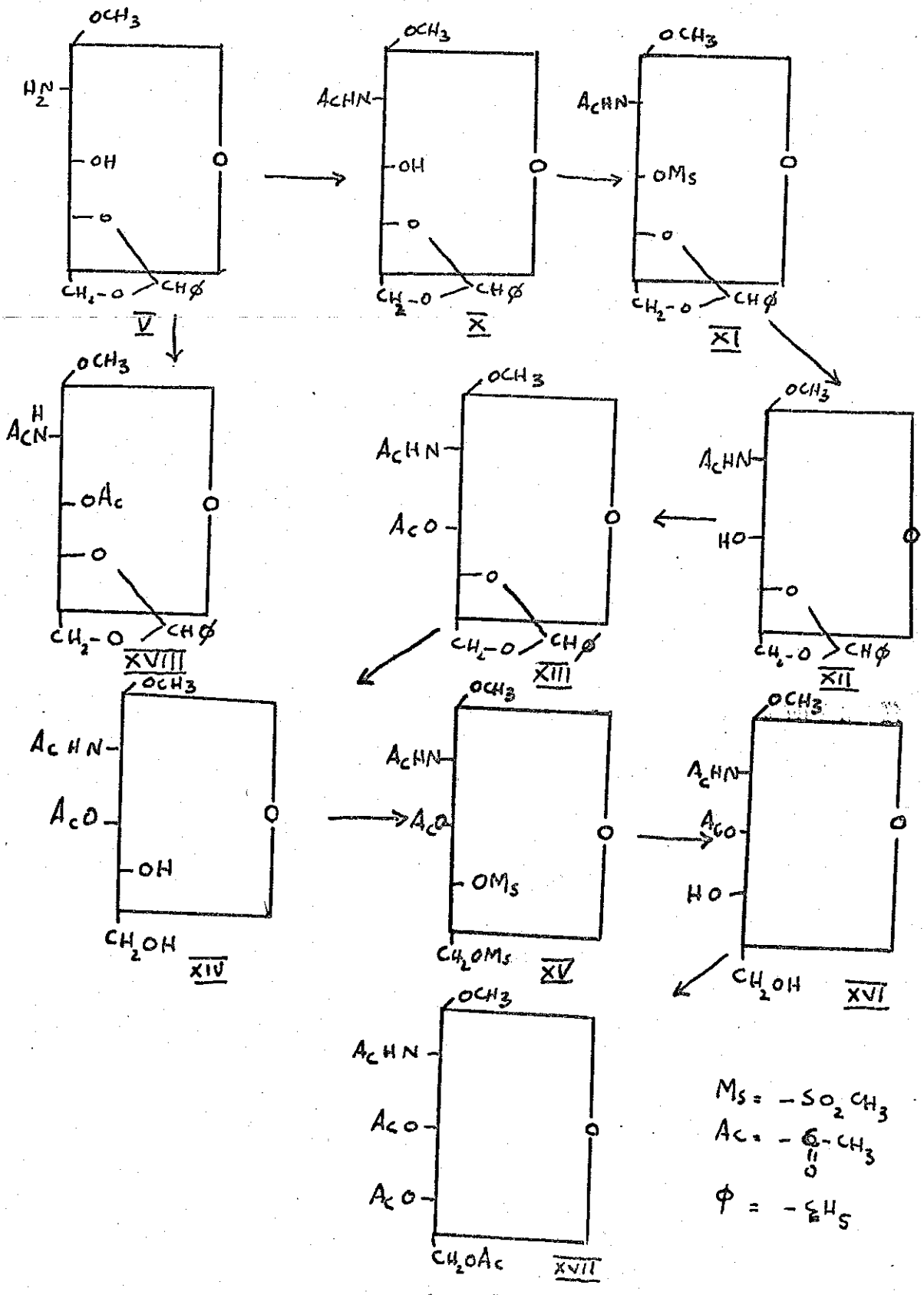
The elimination of the sulphonate under these only weakly basic, and aqueous conditions apparently avoids another side reaction, that occurs, when oxazolines are formed under strongly basic conditions (sodium ethoxide). Taguchi and Kojima (23) found that then the formation of oxazoline from DL-trans-benzamido cyclohexyl toluene-p-sulphonate was accompanied with formation of an epimine, as shown in the following formulas:



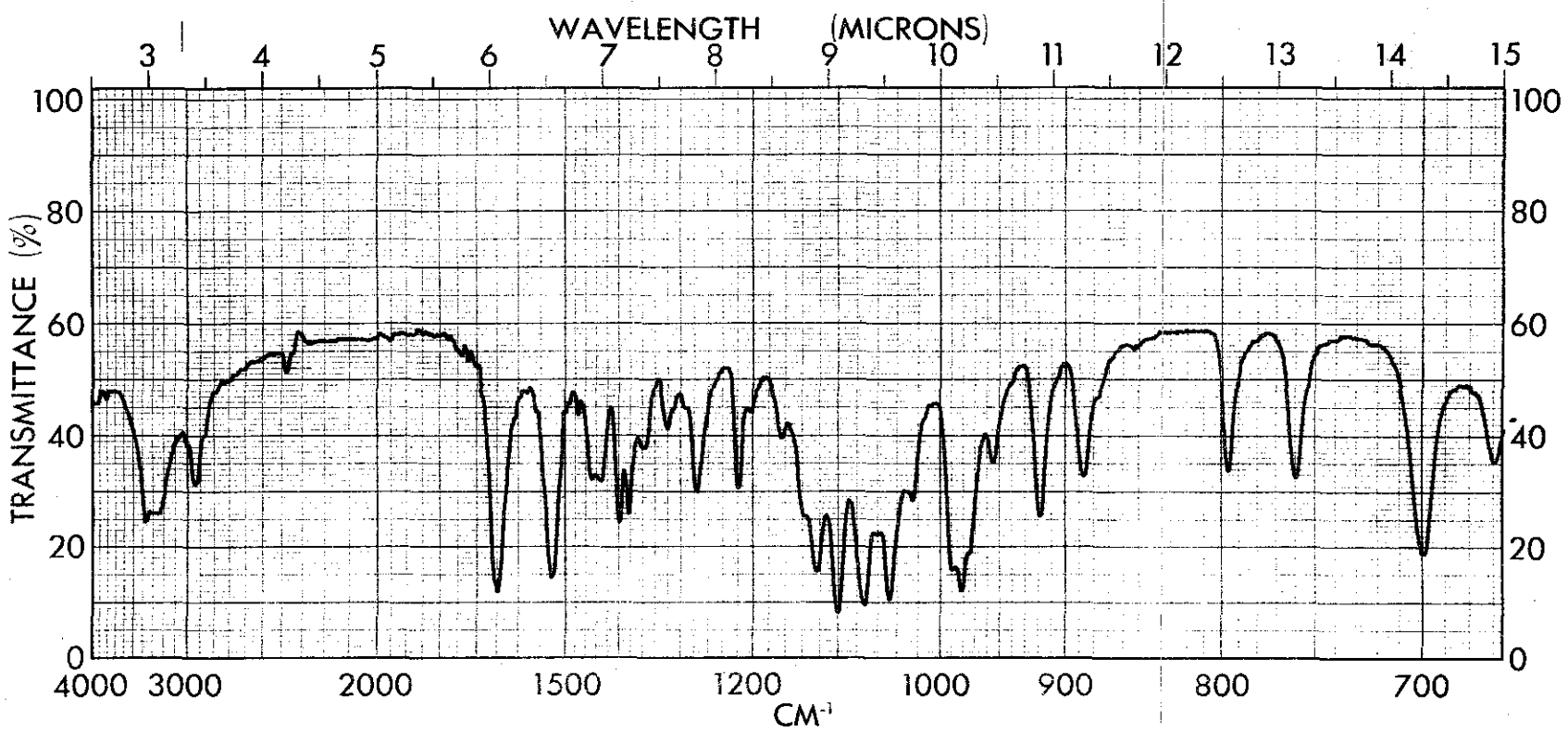
Buss, Hough and Richardson (17) have found similar results in the carbohydrate series. Along with the oxazoline [designated as methyl-4,6-O-benzylidene 3,2 dideoxy 2,3-(2 methyl-1 oxa-3 azo-prop-2-eno)-α-D-manno-pyranoside in their work] they found methyl 2,3-acetyl epimino-4,6-O-benzylidene 2,3-dideoxy-α-D-mannopyranoside, when treated the methanesulphonate (XI) with sodium ethoxide. Under the conditions of our reaction we did not detect the formation of such an epimine, so we have successfully avoided this difficulty at the same time.


OCH3
OH
HO
OH
CH2OH
I
OCH3
OH
HO
O
CH2-O
CHφ
II
OCH3
OMs
MsO
O
CH2-O
CHφ
III
OCH3
O
O
CH2-O
CHφ
IV
OCH3
H2N
OH
O
O
CH2-O
CHφ
V
OCH3
BzHN
OH
O
O
CH2-O
CHφ
VI
OCH3
BzHN
OMs
O
O
CH2-O
CHφ
VII
OCH3
N
φC
O
O
O
CH2-O
CHφ
VIII
OCH3
BzHN
HO
O
O
CH2-O
CHφ
IX
Bz = -C(=O)-C6H5
φ = -C6H5
Ms = -SO2CH3

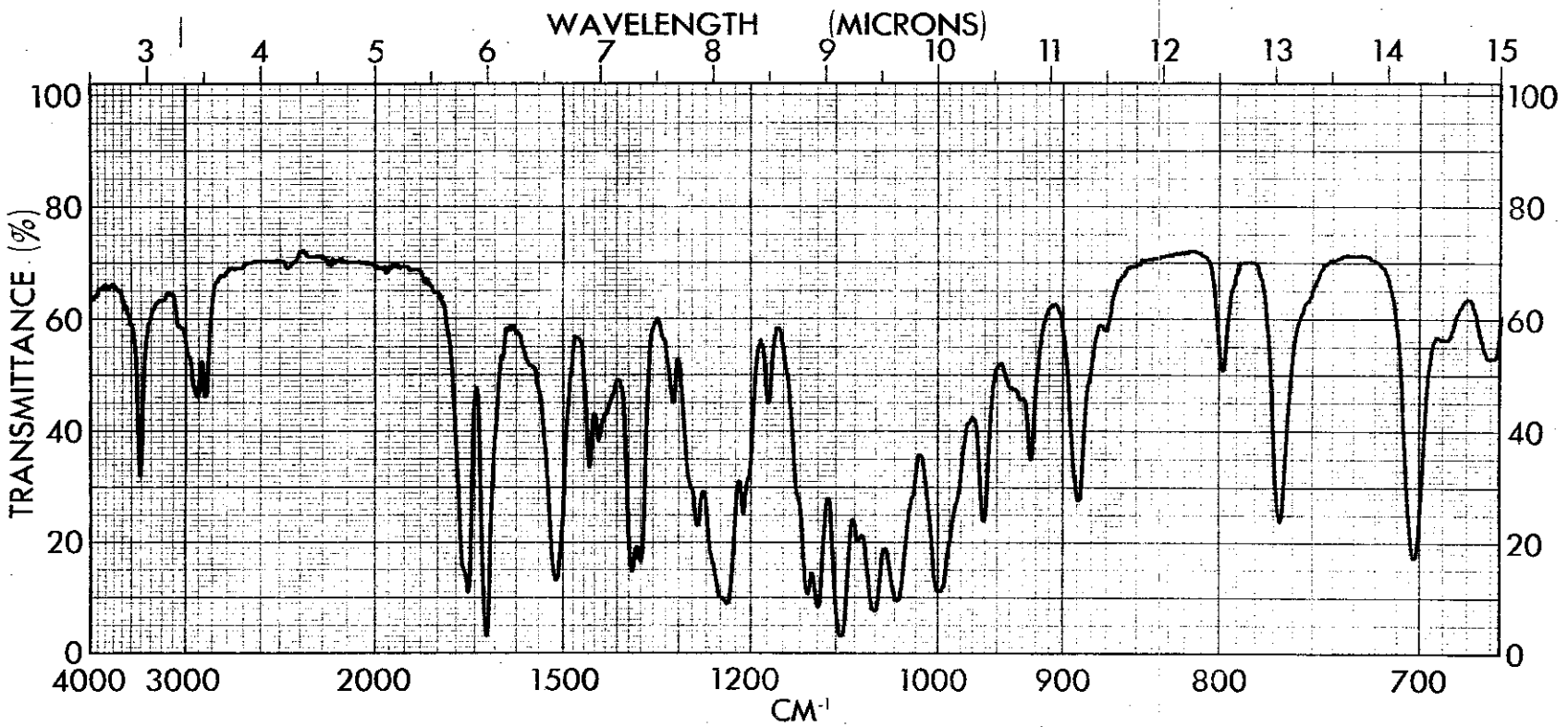
Scheme II


OCH3
H2N
OH
O
CH2-O
CHφ
V
AcHN
OMs
X
XI
H
AcN
OAc
XVIII
AcO
XIII
HO
XII
OH
CH2OH
XIV
CH2OMs
XV
XVI
CH2OAc
XVII
Ms = -SO2CH3
Ac = -C(=O)-CH3
φ = -C6H5

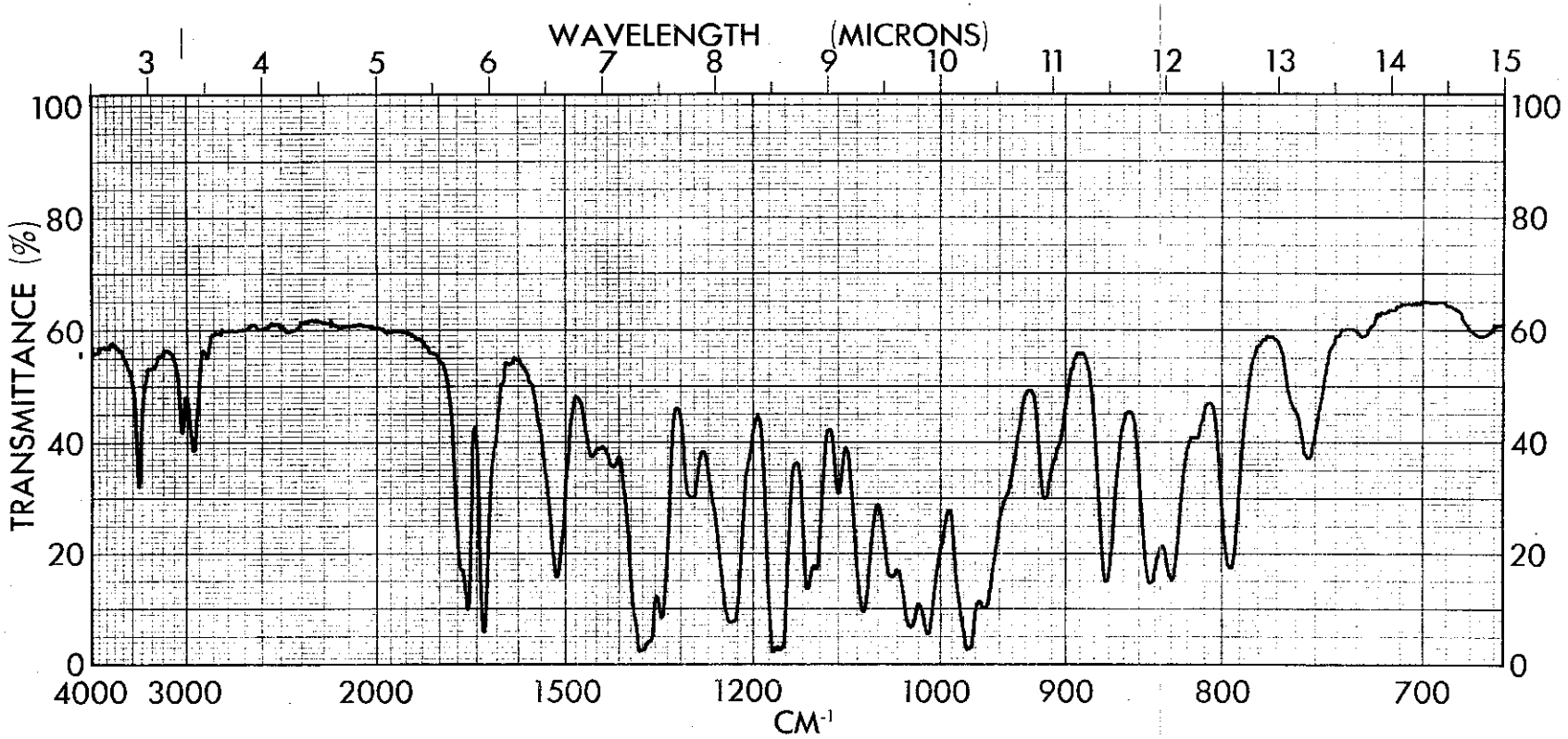
Scheme III



METHYL-2-ACETAMIDO-4,6-O-BENZYLIDENE-α-D-MANNOPYRANOSIDE.



METHYL-2-ACETAMIDO-3-O-ACETYL-4,6-O-BENZYLIDENE-α-D-MANNOPYRANOSIDE



METHYL-2-ACETAMIDO-3-O-ACETYL-4,6-O-MESYL-α-D-MANNOPYRANOSIDE

CHAPTER III

EXPERIMENTAL

Thin layer chromatography was extensively used for the identification of the compounds. The compounds were spotted on a thin layer chromatographic plates which had been coated with the mixture of 32 per cent silica gel, 3 per cent zinc silicate phosphor, and 65 per cent water. The plates were dipped in selected mixtures of ethanol and chloroform. The developing solution was allowed to migrate for about forty-five minutes. The plate after being taken out of the developing mixture was allowed to get dry and then sprayed with a mixture of 10 per cent H_2SO_4 and 90 per cent methanol. The plate was then heated in an oven at about 160°C for about fifteen minutes. In this way carbohydrates are revealed as black spots on the plate.

Since all aminosugars possess asymmetric centers, they are optically active materials. Hence optical rotation is a useful property in their identification and characterization. The polarimeter was used to measure the angle of rotation of the plane of polarized light, using a sodium-vapour lamp. The observed rotation varies with the nature of the substance, its concentration, and the solvent used. The specific rotation, (α), was calculated using the following formula:

$$(\alpha)\ {}^{t}_{\lambda} = \frac{\alpha \times 100}{l \times c}$$

α, is observed angle of rotation.

c, is concentration of the substance in grams of solute per 100

millilitres of solution.

l, is light path in decimeters.

t, is temperature and

λ, is wavelength of the light used (24).

For the determination of melting points a Thiele tube was used, which was found satisfactory for rapid and accurate determinations. All melting points that are reported are uncorrected.

Methyl-4,6-O-benzylidene-∝-D-glucopyranoside (II). A mixture of 120 gms. of methyl glucoside, 90 gms. of freshly fused and powdered zinc chloride, and 300 mls. of benzaldehyde was shaken in a flask for 40 hours. The mixture was poured slowly over 2.5 litres of cold water and the mixture was refrigerated overnight. Petroleum ether (150 mls.) was added with stirring and the crystals were filtered. Washed twice with 200 mls. of cold water, twice with 200 mls. of petroleum ether and again twice with 200 mls. of cold water. The product, after drying overnight in air, was dried in oven at 70°C. Recrystallized from chloroform-ether. M. P. 163° - 164°C; yield 115 gms. (70%). Rotation $[\alpha]_D^{25^\circ}$ + 110° (C 2, chloroform).

Methyl-4,6-O-benzylidene-2,3-dimethanesulphonate-∝-D-glucopyranoside (25) (III). Methanesulphonyl chloride was added slowly to a solution of compound (II), 70 gms. in 200 mls. of pyridine, cooled in ice, and the mixture was kept at 0°C for about 20 hours. The mixture was poured over a mixture of ice and water. Filtered the crystals. Recrystallized from chloroform. Yield 103 gms. (98%), M. P. 188° - 189°C. Rotation $[\alpha]_D^{25^\circ}$ + 49° (C 2, chloroform).

Methyl-2,3-anhydro-4, 6-O-benzylidene-∝-D-allopyranoside (IV). 74 gms. of compound (III) in 1500 mls. 1,2-dichloroethane was cooled in ice and a cold solution of 19.6 gms. of sodium dissolved in 450 mls. of methanol was added. The mixture was kept refrigerated for 3 - 4 days with occasional shaking, and then at room temperature for 1 - 2 days. The solution was diluted with water; the dichloroethane layer was separated, and the aqueous layer was extracted with additional portions of dichloroethane. The combined dichloroethane solutions were washed with water, dried over

$CaCl_2$ and concentrated under reduced pressure. The product crystallized readily and was filtered and washed with ether. Recrystallized from chloroform-ether. Yield 30.2 gms. (92%). M. P. 200° - 201°C. Rotation $[\alpha]_D^{25°}$ + 140° (C 2, chloroform).

Methyl-2-amino-4, 6-O-benzylidene-α-D-altropyranoside (V). 2 gms. of the anhydro sugar (IV) was heated with aqueous ammonia (50 mls.) in a sealed tube at 100°C in an oil bath for 30 hours. Long needles crystallized on cooling. Filtered and washed with water. Recrystallized from water. Yield 1.8 gms. (85%). M. P. 168°C. Rotation $[\alpha]_D^{25°}$ + 104.7° (C 1.35, chloroform).

Methyl-2-benzamido-4, 6-O-benzylidene-α-D-altropyranoside (VI). Benzoic anhydride (3.7 gms.) was added to a suspension of compound (V) (4.15 gms.) in warm ethanol (105 mls.), and the resulting solution kept at room temperature for about 20 minutes. Concentration afforded a syrup which crystallized on addition of petroleum ether. Filtered the product and recrystallized from ethanol and petroleum ether. Yield 5.2 gms. (91%). M. P. 163° - 164°C. Rotation $[\alpha]_D^{25°}$ - 1° (C 1.0, chloroform).

Methyl-2-benzamido-4, 6-O-benzylidene-3-O-methanesulphonyl-α-D-altropyranoside (VII). Methanesulphonyl chloride (0.45 mls.) was added to a solution of compound (VI), (2.1 gms.), in pyridine (7 mls.) and resulting solution kept refrigerated for two days. Poured the mixture over cracked ice. Filtered the product and recrystallized from ethanol and petroleum ether. Yield 1.8 gms. (74%), M. P. 148°C. Rotation $[\alpha]_D^{25°}$ - 11° (C 2.3, chloroform).

Methyl-4, 6-benzylidene-2,3-oxazoline-α-D-mannopyranoside (VIII), 5 gms. of the methanesulphonyl compound (VII) was heated under reflux overnight (about 40 hours) with 5 gms. of anhydrous sodium acetate in 250 mls. of anhydrous ethanol. The solution was evaporated, the residue treated with water, and extracted with diethyl ether. Then the ether phase was evaporated and the residue was recrystallized from ethanol. Yield 3.3 gms. (83%). M. P. 151° - 152°C.

Methyl-2-acetamido-4, 6-O-benzylidene-2-deoxy-α-D-altropyranoside (26) (X). 20 gms. of compound V were dissolved in 350 mls. of ethyl alcohol. 7.2 mls. of acetic anhydride were slowly added to the solution. Let the mixture stand for 10 - 15 minutes at room temperature and then added 7.2 mls. of absolute pyridine. After this another batch of 7.2 mls. of acetic anhydride was added and again 7.2 mls. of anhydrous pyridine were added. Evaporated the mixture to dryness, under vacuum. The residue was taken up in diisopropyl ether and shaken for some time and filtered. The crystals were recrystallized from isopropyl alcohol and heptane. Yield 20.1 gms. (87%). The product was dried in vacuum at 80°C, because it readily tends to form hydrate. M. P. 190° - 191°C. Rotation $[\alpha]_D^{25^\circ}$ + 64° (C 1.0, chloroform).

Methyl-2-acetamido-4, 6-O-benzylidene-3-O-methanesulphonyl-2-deoxy-α--D-altropyranoside (17), (XI). 10.5 gms. of the acetamido derivative (X), were dissolved in 24 mls. of pyridine and the solution maintained at -5°C for some time. 2.0 mls. of methanesulphonyl chloride were added dropwise to the solution which was kept cold in ice-salt bath. The mixture was kept for 48 hours at 5°C. Then it was poured over a mixture of cracked ice

(150 gms.) and 300 mls. of chloroform. Shook the mixture thoroughly and made the water layer acidic (pH 2) to neutralize the pyridine. Removed the chloroform layer and washed it with 10% potassium bicarbonate solution and then washed it twice with water. Evaporated the chloroform layer to dryness and recrystallized the product from absolute ethanol-hexane. Yield 8.3 gms. (66.3%). M. P. 143° - 145°C. Rotation $[\alpha]_D^{25^\circ}$ + 64° (C 0.7, chloroform).

Methyl-2-acetamido-4, 6-0-benzylidene-2-deoxy- α-D-mannopyranoside (XII). A solution of 8.2 gms. of the methanesulphonyl derivative (XI), 12.0 gms. of potassium acetate in 370 mls. of methyl cellusolve (2-methoxyethanol) containing 5% water, was refluxed for 2 days in a 100 mls. flask. After cooling, the solvents were removed by evaporation under reduced pressure. While the solution was being concentrated, it turned into a jello-like substance to which water was added (twice) and evaporated. The third time when water was added the product crystallized out. It was filtered and thoroughly washed with water. Recrystallized from warm dioxane - diisopropyl ether. Yield 5.3 gms. (81.4%). After drying in vacuum dessicater the M. P. came to 225° - 228°C. Rotation $[\alpha]_D^{25^\circ}$ - 11° (C 1, chloroform).

Calculated: 59.24% C 7.19% H 4.19% N 29.38% O

Found: 59.24% C 6.43% H 4.47% N

Methyl-2-acetamido-3-0-acetyl-4, 6-0-benzylidene-2-deoxy-α-D-~~altro~~ manno pyranoside (XIII). 4.0 gms. of the compound (XII) were dissolved in 20.0 mls. of anhydrous pyridine. Acetic anhydride (4.0 mls.) was added to the mixture and the solution allowed to stand for a period of 2 - 3 days at room temperature. The product crystallized out on the addition of diisopropyl ether. Recrystallized from isopropyl alcohol. Yield 3.8 gms.

(90.4%). M. P. 205° - 208°C. Rotation $[\alpha]_D^{25°}$ -3.5° (C 1, $CHCl_3$)

Calculated: 59.17% C 6.34% H 3.84% N 30.65% O

Found: 57.71% C 6.42% H 4.08% N

Methyl-2-acetamido 3-0-acetyl-2-deoxy- α-D-mannopyranoside (XIV). 3.8 gms. of the compound (XIII) were dissolved in 15.2 mls. of glacial acetic acid and then 15.2 mls. of distilled water was added. The mixture was immediately concentrated under reduced pressure by adding water intermittently (3 times). It was further evaporated, 3 times, with absolute ethyl alcohol and toluene to ensure complete dryness. The product came out in the form of a foam which on exposure to air became gummy. Hence it was not possible to obtain it in the crystalline form. Checked its purity by thin layer chromatography and found pure and different from its precussor.

Methyl-2-acetamido 3-0-acetyl-2-deoxy-4, 6-0-methanesulphonyl- α-D-mannopyranoside (XV). To the gummy product (XIV) anhydrous pyridine (1.5 mls.) was added and the solution maintained at ~5°C for some time. Then 0.4 mls. of methanesulphonyl chloride was slowly added to the solution which was kept cold in ice-salt bath. The whole mixture was allowed to stand for 48 hours at 5°C. The mixture was poured over chloroform-ice mixture. The water layer was made acidic and the chloroform layer separated, which was washed thoroughly with 10% potassium bicarbonate solution and then with water. Evaporated the chloroform layer to dryness. To ensure complete dryness it was evaporated three times azeotropically with anhydrous ethyl alcohol/benzene mixture. Recrystallized from isopropyl alcohol. Yield 3.0 gms. (69%). M. P. 120° - 125°C. Rotation $[\alpha]_D^{25°}$ + 35.5°

(C 1, chloroform).

Calculated: 36.02% C 5.35% H 3.24% N 40.60% O 14.80% S

Found: 36.25% C 5.54% H 3.55% N

Methyl-2-acetamido-3,4,6-tri-O-acetyl talosaminide (XVII). 2.7 gms. of the dimesyl compound XV and 7.5 gms. of potassium acetate were refluxed in 170 mls. of Methyl Cellosolve with 5% water for about 36 hours. The solvent was evaporated with intermittent addition of toluene. The dry residue was taken up in 20 mls. of pyridine and acetylated with 10 mls. of acetic anhydride. The mixture was allowed to stand for two days at room temperature, poured on ice and HCl mixture and the compound extracted with $CHCl_3$. The solution was then filtered through silica gel, prepared by Davison Chemical, grade 923, code no. 923-08-08-226, and the compound eluted by ethyl acetate. After evaporation the product was subjected to preparative layer chromatography, using pre-coated plates made by E. Merck A. G. Darmstadt (Germany), and $CHCl_3$/ethyl acetate 3:2 and some toluene. The separation was complete after three developments (as shown later by a thin layer chromatogram). Two zones were faintly visible and were marked, cut out and eluted with isopropanol. The faster moving component was taken up in ethyl acetate. Attempts to crystallize it were unsuccessful. The specific rotation, $[\alpha]_D^{27^\circ}$ + 44° (C 2.8, chloroform) is 17 degrees lower than the reported value (3). This is explained by the fact that the product contained some solvent, as it was syrupy.

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