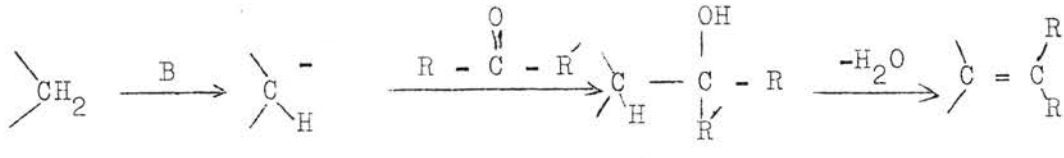
CHAPTER IV

Discussion

Condensation of Aldehyde and Rhodanine

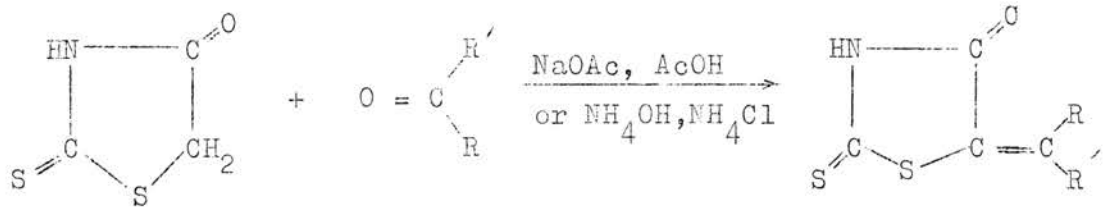
The methylene carbon atom at the 5-position of rhodanine causes nucleophillic activity. The reaction frequently occurs in the presence of a base and the anion of the thiazolidine ring is the attacking species. The ease of formation of the anion, and hence the degree of the nucleophillic activity, is dependent not only on the electron-withdrawing effect of the adjacent carbonyl group, but also on the presence of another electron-withdrawing group, the thionyl, attacking the 2-carbon atom (91).

Rhodanine can undergo aldol condensation with carbonyl of aldehyde or ketone, generally followed by a loss of water. The product of the reaction contains an α, β-unsubstituted carbonyl group. The reaction is classified as a variation of Perkin - Condensation, in which a base (the salt of an inorganic acid or the salt of a carboxylic acid) is used, and the reaction is accompanied by the formation of carbanion :



In general, the condensation reaction of rhodanine with aldehydes or ketones, producing 5-substituted rhodanine, requires

a condensing agent. Anhydrous sodium acetate in glacial acetic acid (60) or ammonium chloride in ammonia (81) are usually used, and the reaction can be shown as follows :



There is evidence that the aldol reaction of rhodanine derivatives is reversible, since the product, if treated with an alkali, gives the odor of aldehyde (99). The reverse aldol reaction is complicated by the decomposition of the rhodanine nucleus by the alkali.

In addition, several compounds can also be used as condensing agents, namely sodium hydroxide in ethanolic solution (76), sodium ethoxide in ethanolic solution (77), anhydrous sodium acetate in acetic acid (60), anhydrous sodium acetate acetic anhydride and acetic acid (78), ammonium hydroxide in ethanolic solution (82), diethanolamine(83), piperidine (84,35), and sulfuric acid (80).

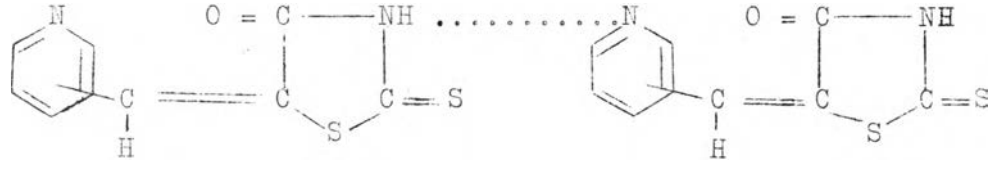
In the preparation of the 5-arylmethylene rhodanine derivatives reported here, only anhydrous sodium acetate, with glacial acetic acid as a condensing agent, was used. The procedure involved dissolving the rhodanine in glacial acetic acid by warming the mixture in a water-bath, then adding 3 times of

mole of anhydrous sodium acetate. After the salt had dissolved, an equimolar of aldehyde was added to the rhodanine. The mixture was refluxed for 30-60 minutes. Some preparations formed crystal products during the reflux. However, after cooling the refluxed mixture to room temperature, most preparations formed crystal products. The formation of crystal products during refluxing indicated the ease of condensation between rhodanine and aldehyde in the presence of sodium acetate and acetic acid. In order to gain more crystals, the mixture must be kept in the refrigerator overnight. The crystals were isolated by filtration and washed with water to remove the excess acid. In order to maximize the yield of the product, the filtrate was combined with the washed water and poured into another amount of water which gave more crystals after cooling in the refrigerator. Purification was generally done by recrystalization, but the first crop crystallized from the reaction mixture was usually pure enough for producing subsequent reactions.

The product obtained was dried in an oven at 50°C with reduced pressure. The total yield obtained from this process was between 50-97 %, and the average percentage yield was 84 %

The 5-substituted rhodanine derivatives appeared as needle shaped crystals with colours ranging from yellow to red, and had a high melting point (over 200°C), except for the 5(2,6-dichlorobenzylidene) rhodanine. All the derivatives of pyridine carboxal-

dehydes had very high-melting points and exhibited characteristic insolubility in a common organic solvent. Allan et al.(132) have ascribed these properties to the amphoteric nature of the condensation adducts which permit intermolecular salt formation between the basic nitrogen of the pyridine ring and the acidic imino hydrogen of the rhodanine, as shown in the following illustration :



They have also found that substitution of the imino hydrogen atom of the rhodanine ring by a phenyl group blocks the possibility of intermolecular ionic bonding, and restores the normal solubility pattern of the condensation adducts lowering the melting point.

In addition to establishing the amphoteric property of rhodanine derivatives, Sandel and Neumayer(135) have reported that 5-(p- diethyl aminobenzylidene) rhodanine exhibits an ampholyte property :

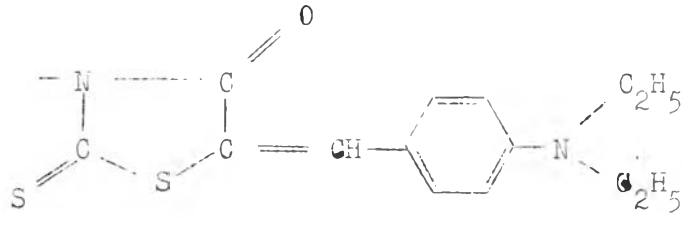
$$HR - H^+ \rightleftharpoons HR + H^+$$

(very pale yellow) (pink)

$$HR \rightleftharpoons R^- + H^+$$

(pink) (yellow)

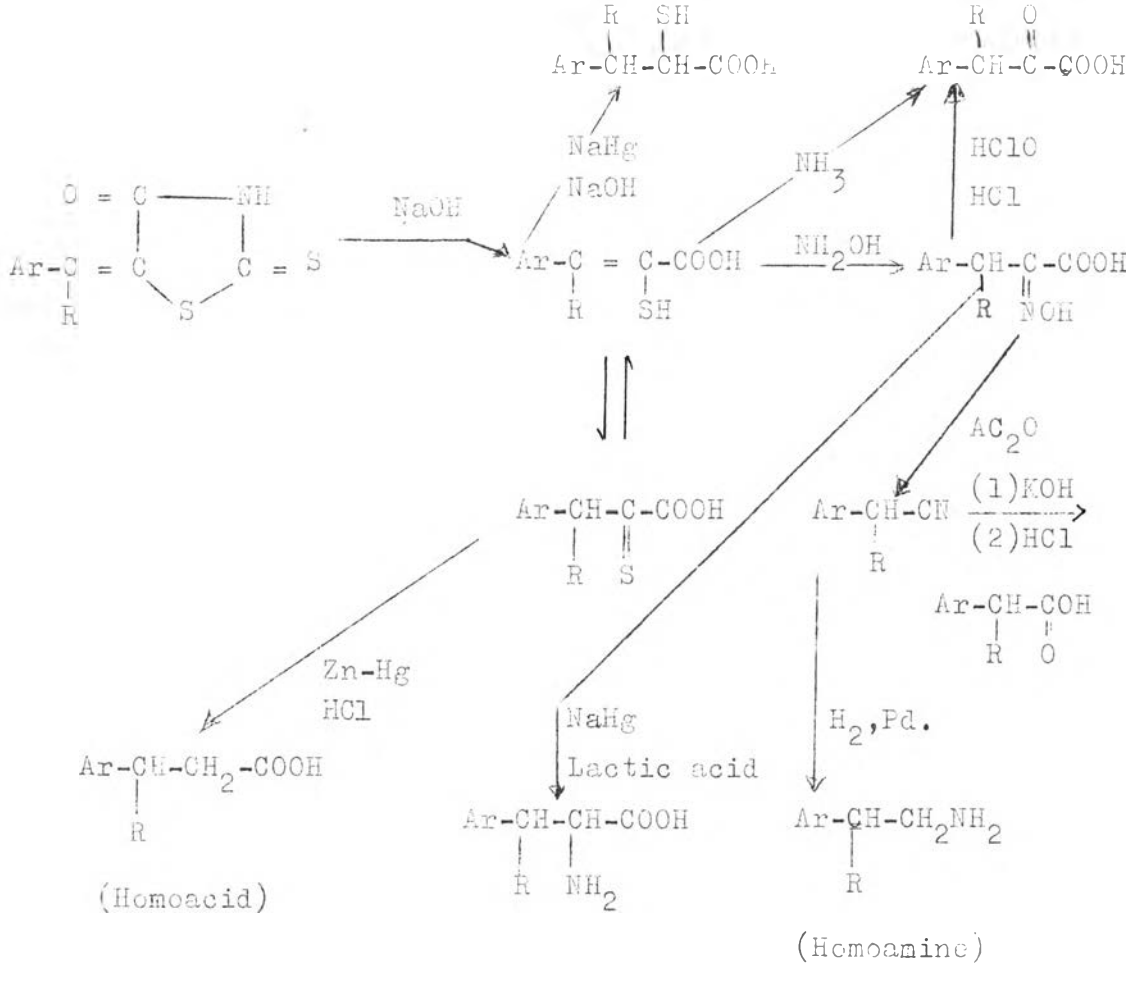
R =



Structurally speaking 5-(p-dimethylaminobenzylidene)rhodanine could be considered as an ampholyte as well. In fact both analogues, the dimethyl and diethylaminobenzylidene rhodanine have been used in chemical quantitative analysis (105).

Starting with the condensation products (5-arylmethylene rhodanine), many types of compounds can be made. The alkali hydrolysis of 5-arylmethylene rhodanine leads to a common result, the production of α-mercaptoacrylic acids, which have been utilized in a series of reactions, as shown in the following scheme:

One of the most interesting series was reported by Julian and Sturgis(60) on homoamine and homoacid :



All of these acids and amines are more or less important in making both medicinal compounds and natural products such as adrenergic agents, antiinflammatory agents, antihistamine and amino acids..

Preparation of α - D-Acetobromoglucose

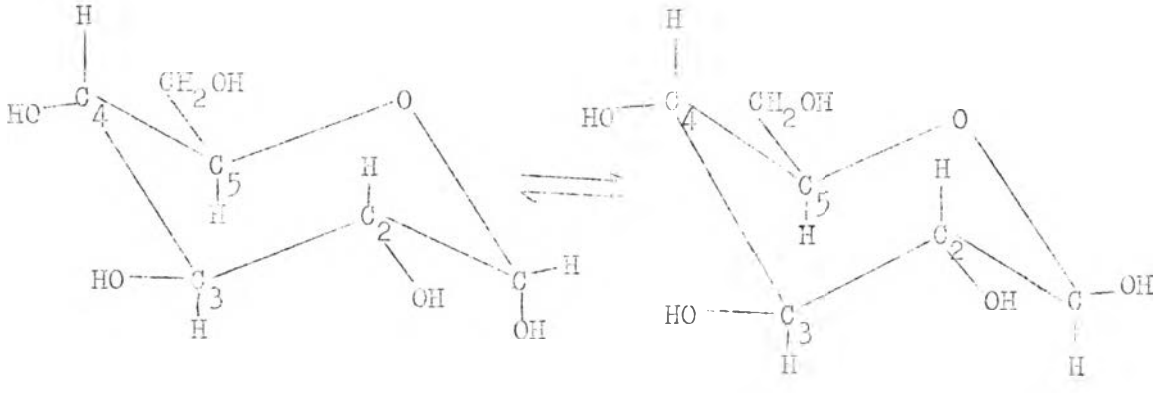
In general, the method for preparing glucosyl halide derivatives, including acetobromoglucose, involves the replacement of an acyloxyl group by a halogen at the reducing carbon atom. The classical procedure is to make the acetobromoglucose by treating the sugar acetate with a solution of hydrogen bromide in either glacial acetic acid or acetic anhydride. (136,137) However, there are difficulties in preparing and handling the solution if it is prepared in this way. A more satisfactory method is the one proposed by Barczai-Martos and Korozy(138), in which the hydrogen bromide is generated in situ. A further improvement has been worked out by Lemieux (134), making the procedure more convenient and giving an excellent overall yield.

For the purposes of the present study acetobromoglucose was prepared in accordance with the Lemieux method, but with certain modifications as described in the experimental part of Chapter III (see p.p. 30) Previous studies have shown that in preparing acetobromoglucose, especially at the grinding and crystalizing stage, an anhydrous condition is essential. Indeed, in the relatively high humidity of the climate of Thailand, decomposition to a black resinous mass may occur unless $CaCO_3$ is used as a stabilizer. So following the recommendation of Isbell and Frush(139), $CaCO_3$ was used as a stabilizer for the preparation of acetobromoglucose, since the carbonate may neutralize the acids

formed by incipient decomposition and stabilize the halide.

There are several points of view as to how this preparation should be carried out. With respect to anomerization, it is known that free sugars exist in a crystalline state in the form of a lactol ring. The carbon of the "masked" carbonyl group is known as the anomeric center and is asymmetrically substituted. When a crystalline sugar is dissolved in water and a stage of equilibrium is reached, a lactol ring forms an open chain of either an aldehyde or a keto form of the sugar. On relactolization, the sugar may either revert to the original form, but opposite configuration, at the anomeric center (which is known as the anomer) or it may develop a different anomeric structure. Thus the sugar exists in a dynamic equilibrium both in the case of anomerization and ring isomerization. Such change in optical rotation which occurs in the process of equilibrium has been termed "mutarotation"

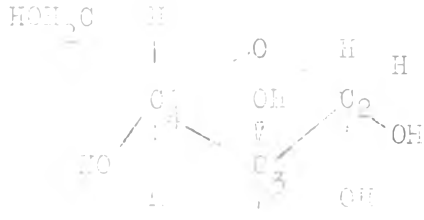
The stereochemistry of the pyranose can best be appreciated from the conformational formula XXII (α form) and XXIII(β-form)



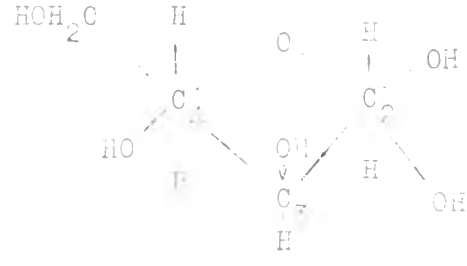
XXII (α- form) XXIII (β- form)

The preferential stability of the chair form is due to the fact that the large hydroxymethyl group assumes an equatorial orientation, which is linked at C5 of all D-Sugar conformation having the same chair pattern. The β-D anomer form has the 1-hydroxy group in equatorial orientation, whereas the α-D-form has this group in an axial orientation and is destabilized by the interaction of the hydroxy group with the two axial hydrogens.

The α-D-anomer shows a cis relationship between the hemiacetal hydroxy group at the carbon atom 1 and the hydroxy group at the carbon atom 2, whereas the β-anomer has a trans relationship between these two hydroxy groups. The two following figures representing a view from the carbon 2 directly towards the carbon 1, show the angular relationship of the hydroxy group attached to the carbon 2(solid line) to that of the group attached to the carbon 1(dotted line)



α-anomer, cis;



β-anomer, trans.

In addition, Kabayama and Patterson (140) have also pointed out that an aldopyranose structure fits into the tridymite structure of water most effectively when the hydroxy group at C-1 is equatorially oriented.

The hydroxy groups of a carbohydrate material, including D-glucose, react readily with acetic anhydride in the presence of an acidic catalyst or a basic catalyst such as sodium acetate or pyridine

$$\text{R-C-OH} + Ac_2O \xrightarrow{\text{Catalyst}} \text{R-C-OAc} + HOAc$$

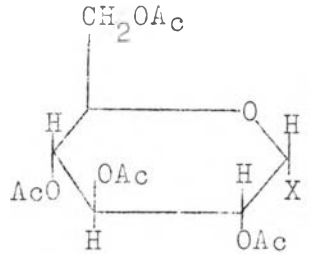
The acidic catalysts may be zinc chloride, hydrogen chloride, sulfuric acid, or perchloric acid. The stability of an alditol allows it to be acetylated under almost any conditions. The sugars present a different problem in that they exist in solution as equilibrium mixtures of tautomers that are acetylated as such in the reaction mixture. The production of the acetylated β-D-anomer is favoured at high temperature using sodium acetate as a

catalyst (141). Acetylation of α- or β-D-glucose with acetic anhydride and pyridine at 0°C occurs without anomeric change(141). At higher temperature and in the presence of acidic catalysts, such as zinc chloride or sulfuric acid, an anomerization equilibrium is set up favouring the α-D forms(141). In addition, the ring size of the cyclic acetates formed by the common acetylation procedures normally has a pyranoid structure.

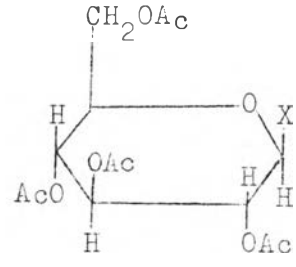
It has been reported that the composition at equilibrium of the acetylation reaction is approximately 87 % of the α-form and 13 % of the β-form, using either sulfuric acid or perchloric acid as a catalyst (142). Since in this study the acetylation reaction was obtained using perchloric acid as a catalyst in common acetylation (refer to Painter's report), it can be said that the unisolated pentaacetate glucose was a predominantly α-anomer.

The D-glucopyranose pentaacetate has been transformed, under a wide variety of conditions, (138, 143-147) to tetra-O-acetyl- α-D-glucopyranosyl bromide, a compound widely known as "acetobromoglucose" The reagent in all cases is essentially hydrogen bromide in glacial acetic acid and reacts readily with both anomeric forms of D-glucopyranose pentaacetate. For this reason, a mixture of pentaacetates, which is obtained on the acetylation of D-glucose under acidic conditions, can be used for making the acetobromoglucose (146,138) It will be appreciated that just as the simple glycosides can exist in anomeric forms,

the glucosyl halides can exist in two forms. This is exemplified in the two Haworth formulas XXIV and XXV for tetra-O-acetyl-D-glucopyranosyl halide. With the halides, however, one form is usually considerably more stable than the other.



XXIV α-form



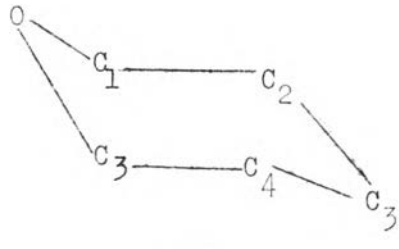
XXV β-form

In most of the halides investigated, the stable form appears to be the α-anomer, with the exception of the derivatives of arabinopyranose, ribopyranose and fructopyranose (148).

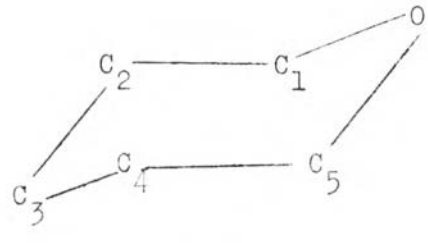
Most of the preparative methods give the stable form of the poly -O-acetylglycosyl halide as the final product, irrespective of the anomeric configuration of the starting material. There is, however, some basis for claiming that the initial product may be either an unstable or stable form of the halide, depending on the configuration of the starting material. It is to be expected that tetra-O-acetyl-β-D-glucopyranosyl bromide will be formed and reversibly converted to the thermodynamically more stable α-anomer in the course of the reaction and will undergo rapid hydrolysis

during the isolation procedure (147, 149). Probably because of the rapid hydrolysis, the β-anomer has never appeared as a reaction product, although it is undoubtely present in the reaction mixture and thus contributes to the loss in yield.

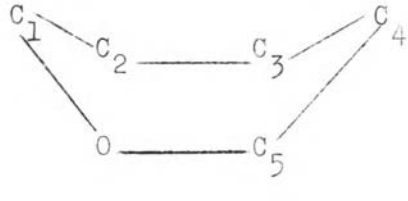
The greater stability of the α-form over the β-form has been explained in the course of conformational studies (150,151). It is thought that the pyranose ring is theoretically capable of eight strainless ring conformations (structure XXVI - XXXIII), giving six "boat" forms and two "staggered" or "chair" forms :



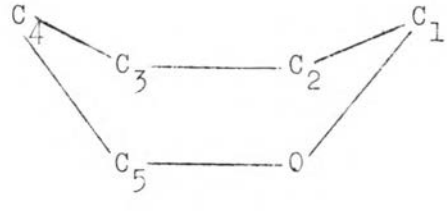
XXVI



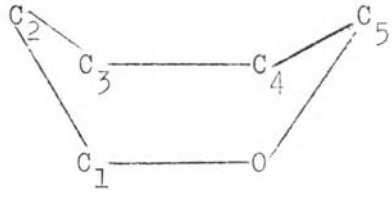
XXVII



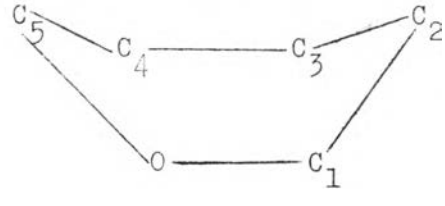
XXVIII



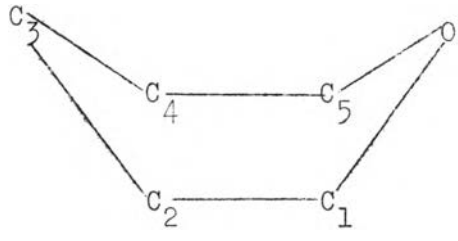
XXIX



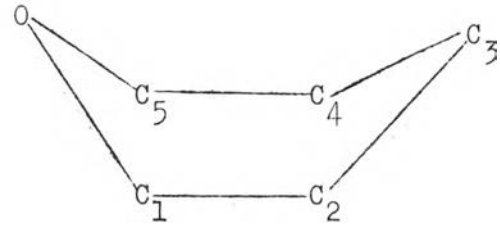
XXX



XXXI



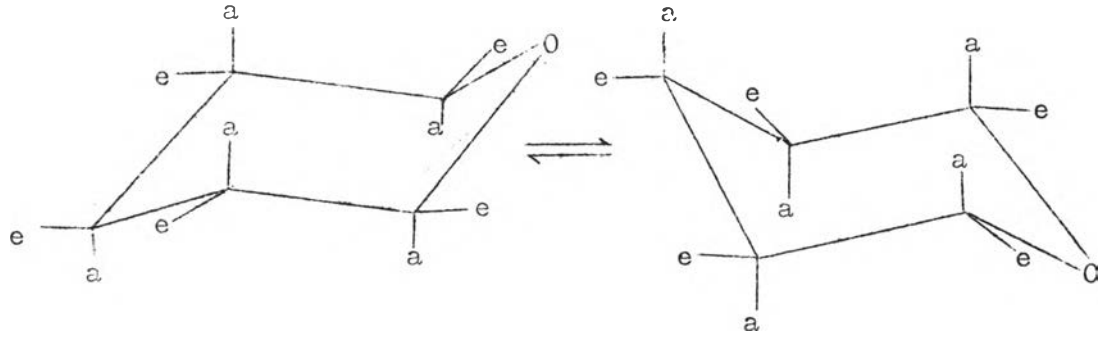
XXXII



XXXIII

The evidence indicates that one of the two chair forms will be favoured and that for most purposes the boat forms can be neglected.

The equilibrium equation between those two strainless chair forms can be drawn as follows :



In the case of glucopyranose, the interaction betwen the groups on carbon 1 and 5 is important and determines the stability of the isomers. The anomer having a stable arrangement of the halogen and a large substituted group at the carbon 5 in the two chair forms can be represented by the following equation :

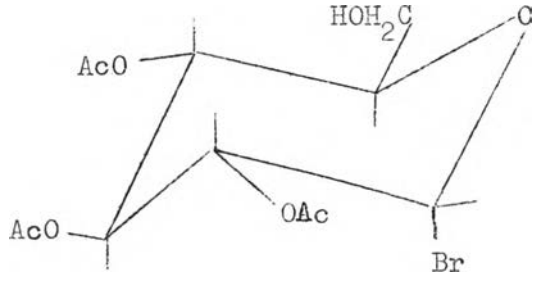
(A) $-X_a, -CH_2OAc_e \rightleftharpoons -X_e, -CH_2OAc_a$

which is more stable than the other anomer represented by

$$(B) \qquad -Xe, -CH_2OAc_e \rightleftharpoons -X_a, -CH_2OAc_a$$

The letters e and a refer to equatorial and axial positions of the substituents respectively. Hence the α-form, where the bromine is axial and the CH_2OAc is equatorial (equation A), is more stable than the β-form in which both groups are either in axial or equatorial positions(equation B)

In addition, the effect on the stability of the isomer due to the interaction between the groups at the carbons 1 and 5 and the ring oxygen is significant, and the anomeric effect will strongly favour the axial product :



Further more, the anomeric effect has been conclusively established, through N.M.R. spectroscopy, by Lemieux and his co-workers (152) who found that the alkoxy, acetoxy and halogen groups prefer the axial to the equatorial orientation when at the anomeric center of an aldopyranose.

In common with other halide derivatives, the order of stability is as follows : fluoride > chloride > bromide > iodide.(148).

The fluorides are extremely stable, and may even be deacetylated without loss of fluorine, giving the corresponding glycosyl fluorides. The iodides, on the other hand, are unstable compounds which even in favourable cases decompose at room temperature within two weeks. The bromides show a reasonable balance between reactivity and instability, and have been by far the most widely used halides for synthetic work.

Preparation of N-Tetra-O-acetyl-β-D-glucopyranosyl-5-arylmethylenerhodanine

Many natural products occur as glycosides, such as the anthocyanine, the cardiac glycosides, the saponins and the hydroxyanthraquinone glycosides. Following the work of Koenigs and Knorr on glycoside synthesis, a large number of compounds of this type have been synthesized by using glycosyl halides. However, more attention has been paid to the synthesis of glycosylamines(N-glycosides), particularly the biologically important nucleosides.

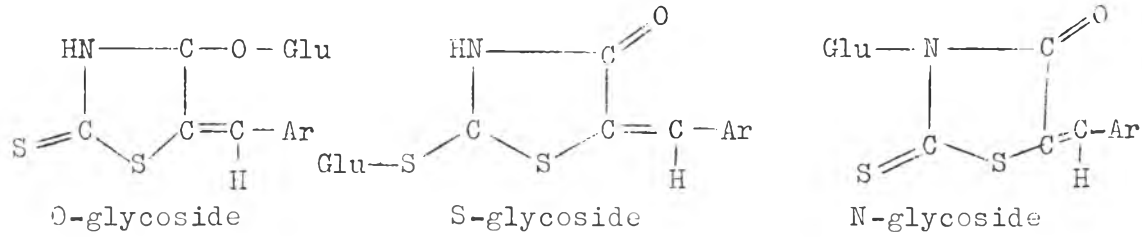
Tetra-O-acetyl glucopyranosyl derivatives of rhodanine are prepared following the procedure described by Foye and Tovivich (54). This consists of completely dissolving an equimolar of 5-arylmethylene rhodanine and acetobromoglucose in acetone using a stoppered conical flask. Then 10 % sodium hydroxide solution, as equimolar, is added to the stirring solution. The mixture is stirred at room temperature for 3-4 days. A precipitate of sodium

bromide, at the bottom of the vessel, indicates the reaction has taken place, and by using the thin layer chromatography technique, the stage at which the reaction is completed can be verified. After 3-4 days of stirring, the solution is filtered, and the precipitate can be shown to be a halide, since it produces a positive reaction to silver nitrate test solution in nitric acid solution. The filtrate is evaporated in a rotaevaporator at 50°-60°C under reduced pressure. A yellow precipitate is formed, as is the case for almost all glucosylated rhodanine preparation.

The crude products are purified usually by crystalization from methanol and normally form needle crystals. The yield ranges from 60 % to 92.8 % giving an average yields of 73.8 %

The acetobromoglucose prepared was stabilized by using 1 % $CaCO_3$, but before using this compound, it is necessery to remove the $CaCO_3$ by dissolving the compound in acetone and filtering off the salt.

It can be seen from the structure of rhodanine derivatives that there are three positions which could theoretically be the site of glycosidation - O- glycoside at the 4 position, S-glycoside at the 2 position, and N-glycoside at the 3 position

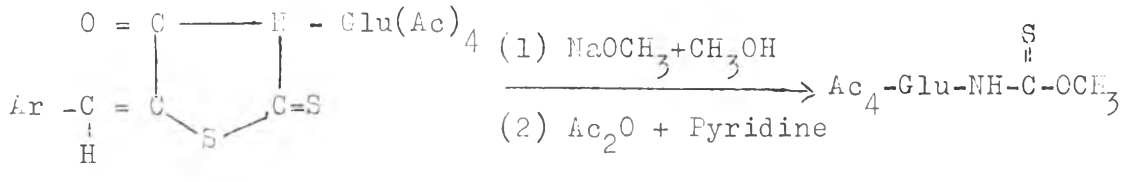


O-glycoside S-glycoside N-glycoside

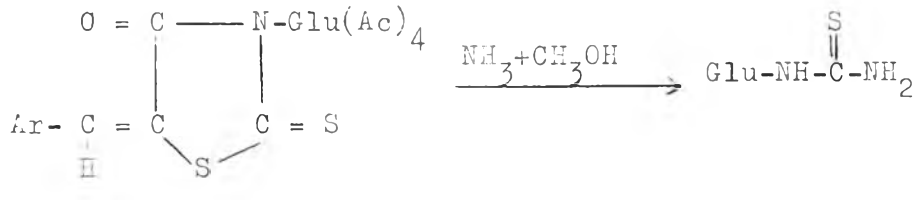
The following tests support the fact that the glycosylated rhodanines are N-glycosides (53,54) :

1. The Chemical Reaction

When the acetylated glycosyl derivative is hydrolyzed with sodium methoxide in methanol, followed by acetylation, the resulting product is N- (tetra-acetyl-D-glycosyl) methylthiocarbamate(53)



In addition, hydrolysis of acetylated glucosyl derivatives with ammonia in methanol gives glucosyl thiourea(54)



Both basic-hydrolysis products support chemically the conclusion that the acetylated glucosyl moiety forms an attachment to the nitrogen atom of the rhodanine ring.

Moreover, it has been found that substitution of the rhodanine ring at the nitrogen atom lowers the melting point

(132) The glycosylated rhodanines obtained here, with a few exceptions, had melting points lower than those of the corresponding aglycones. It can therefore be safely concluded that the glucose moiety forms an attachment to the nitrogen atom of the rhodanine

2. Infra-red Spectrum Analysis

Using the IR. spectra, there is significant evidence to prove that the glycosylated products are in fact N-glycosides. The non-glycosylated rhodanine gives a medium doublet peak between the region of 3400 cm^{-1} and 3100 cm^{-1}, whereas the glycosylated derivatives give a weak singlet peak in the same region. This indicates that the imino hydrogen atom is replaced by the sugar derivative. In addition, two characteristic peaks of non-glycosylated derivatives occur - one between 1250 cm^{-1} and 1100 cm^{-1} (which represents the thionyl group at position 2), and the other between 1760 cm^{-1} and 1650 cm^{-1} (which represents the carbonyl group) -and they remain virtually unchanged after glycosylation. This is also an indication that there is still no substitution either at the thionyl or the carbonyl group.

Another significant feature is that all the reported glycosylated rhodanine derivatives were β-anomer, whereas the acetobromoglucose used was α- anomer. According to Lemieux's paper (153), the reaction of α- acetobromoglucose in glucosidation is a Sn 2 type reaction mechanism. In addition, it was previously suggested

by Hayes and Newth (148) that if the halogen atom is cis to an O-acetyl group at C 2, as the acetobromoglucose is, a reaction with a nucleophillic reagent results in complete inversion of the configuration at C 1. On the other hand, a transdisposition of the halogen with respect to the neighbouring acetyl group prompt the formation of a considerable amount of 1,2-orthoacetate, together with the anomeric α and β glycosides.

Preparation of N-β-D-glucopyranosyl-5-arylmethylene rhodanine

The acetyl groups of sugar, including glucopyranosyl, can be removed by several methods, as shown in the following equations :

$$ROAc + MeOH \xrightarrow{NaOMe} ROH + AcOMe$$

$$ROAc + MeOH \xrightarrow{Ba(OMe)_2} ROH + AcOMe$$

$$ROAc + NH_3 \xrightarrow{MeOH} ROH + AcNH_2$$

$$ROAc + HN(Me)_2 \longrightarrow ROH + AcNMe_2$$

$$2ROAC + Ba(OH)_2 \longrightarrow 2ROH + Ba(OAc)_2$$

$$RNHAc + HOH \xrightarrow{HCl} RNH_3Cl + AcOH$$

In general, the carbohydrate acetates, which are easily purified and interconvertible with the parent substances, are ideal derivatives for the isolation and purification of the sugars. Ester hydrolysis can be carried out using either an acid or a base as catalysts, but bases are more powerful catalysts than acids. Deacetylation with a methanolic solution containing an amount of

sodium (154 - 157) or barium methoxide (158) can be used for transesterification and the effects on the free sugars are only slightly different. A methanolic solution of ammonia (159) ammonolyze acyl groups to form acetamide in the case of glycosides and other sugar derivatives in which the carbonyl group is protected. Methanolic solutions of dimethylamine (160) and other amines can be used in place of ammonia. Although sugars are highly unstable in alkaline solutions, a cold saturated aqueous solution of barium hydroxide has been found very useful, espectially for the O-deacetylation to ketoses (161).

Conversion of tetraacetyl glucopyranosyl of rhodanine to glucosyl rhodanine israther difficult, since the rhodanine nucleus is sensitive to alkaline. Bognar and Wieniawski (53) were unsuccessful in deacetylating their tetraacetylglucosyl rhodanine derivative by alkaline hydrolysis. Saponification with sodium methoxide caused methanolysis of the rhodanine ring, and with barium hydroxide it also failed to give the desired glucoside. In addition, Foye and Tevivich (54) found that by leaving cool ammonical methanol in an open system for 20 hours, the tetraacetylglucosyl derivatives of 5-substituted rhodanine underwent hydrolysis, cleaving the rhodanine moiety from the glucose derivative, and yielding the 5-substituted rhodanine. If the reaction was carried out in a sealed bottle with methanol saturated with ammonia, the product was glucosyl thiourea.

It has been established that alkali hydrolysis cannot be used due to the sensitivity of the rhodanine ring. Foye and Tovivich (54) also failed to condense 5-substituted rhodanine and glucose directly in various solvents, such as dimethylsulfoxide (DMSO), tetrahydrofuran (THF) and methanol. A successful method for deacetylation of the tetra-acetylglucosyl-5-substituted rhodanine has been worked out by using hydrochloric acid in methanol (54)

The procedure involves suspending or dissolving the tetraacetylglucosyl-5-substituted rhodanine in methanol, then adding 2M. hydrochloric acid which is 4 times the amount of mole of the tetraacetylglucoside. The mixture is stirred with a magnetic stirrer at room temperature for 4 days; but in some preparations, the reaction has to be refluxed to get a clear solution, which subsequently becomes a suspension when cooled to room temperature. The completeness of the reaction can be observed by noting the clarity of the solution, or sometimes by using thin layer chromatography to determine the disappearance of the starting compound in the reaction mixture. The reaction mixture is filtered and evaporated under reduced pressure in a rota-evaporator. The residue obtained is purified by using ethanolic water which gives a crude precipitate. A crude yield resulting from this procedure is usually between 71.5 % to 99 %, or in other words a 91 % average yield.

As regards the purification of the final crude product, it is known that glycoside compounds, especially the compounds under this investigation, do not crystalize very easily. Various solvent systems were used for recrystalization of certain derivatives and did not result in satisfactory purification. In addition, fractional recrystalization also resulted in a reduced yield. Another method of isolating sugar derivatives and glucosides is by using column chromatography (162-168). This technique as applied to the isolation of glucosyl derivatives of rhodanine; involves the use of a glass tube column 1 inch in diameter and 30 inches long. Silica gel (230-400 mesh. E-merck) is used as an adsorbent, and is suspended in a solvent (usually ethyl acetate) and poured into the tube up to a height of 15-20 inches. The packed column is then allowed to settle for 12-24 hours so that the adsorbent can become homogeneous. Approximately 1 g of the crude glucoside is then dissolved in a small amount of acetone and ground with a small quantity of the adsorbent to dryness. Subsequently a thin layer of the powder is transferred to the top of the column, and elution of the solvent system is carried out slowly at a rate of 20-40 drops per minute. Since most of the compounds concerned are coloured, several yellow zones can be observed visually and only the main zone, which is strongly adsorbed, remains near the top of the column and is collected.

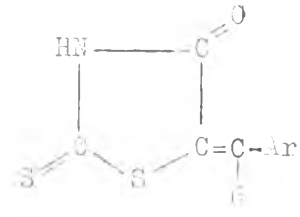
When this technique was used in the present study, it was

found that the fraction collected was of satisfactory purity. No attempt was made to identify and quantitatively determine the other yellow zone. However, once the first zones to settle had been collected and the solvent removed, a measurement of the melting points confirmed that it was non-glucosylated 5-substituted rhodanine.

Since the solvent systems used - ethyl acetate or a gradient mixture of chloroform-methanol, were non-polar systems, it was to be expected that in accordance with the principle of "like dissolves like", the deacetylated compounds (which give 4 polar hydroxy groups at the sugar moiety were more polar than the non-glucosylated and tetra-acetyl glucoside derivatives - also that the deacetylated glucosides would remain strongly adsorbed near the top of the column. In other words, it is fairly clear that deacetylating with hydrochloric methanol produces optimum results.

Infra-red spectroscopy has been used to identify or distinquish between different substances and to determine the structure of a given compound. The discussion given here will be concerned mainly with observations on structure of the compounds. The compounds involved are those with the following features .-

1) 5-arylmethylene rhodanines, consisting of the following major functional groups :



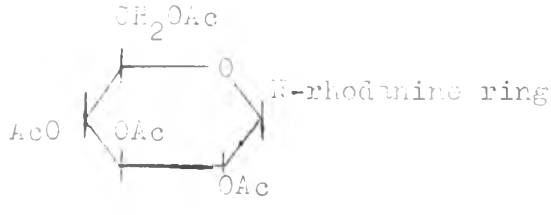
a) carbonyl group (C=O)

b) thionyl group (C=S)

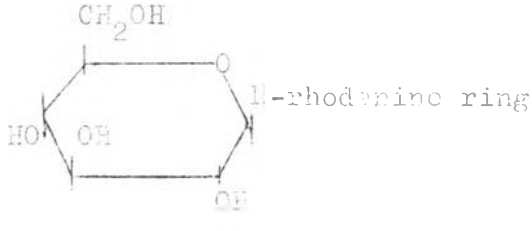
c) imino group (-NH)

and d) aromatic ring

2) Tetra-acetyl-glucosides, having a glucose moiety with a β-linkage and poly-O-acetyl groups:



3) Acetyl-free glucosides, having a poly-alcoholic group instead of a poly acetoxy group.



For the 5-Substituted rhodanines, the carbonyl peak of 4-thiazolidinone is usually found between 1760 cm^{-1} and 1655 cm^{-1} (169). This is a strong, characteristic peak indicating a C=O stretching vibration. It is noticeable that the carbonyl group of the unsubstituted rhodanine shows a strong peak at 1700 cm^{-1}; whereas rhodanine derivatives, a saturated alkyl group in the 5-position does not have a significant effect on the position of the peak caused by the 4-carbonyl group. Unsaturation at the 5-position, however, produces conjugation with the carbonyl group resulting in a bathochromic shift (169)

The thionyl peak (C=S) of the rhodanine derivative is indicated by a strong band between the region 1250 cm^{-1} and 1100 cm^{-1} (169) Since this region is the same as the vibration of C-O and C-N stretching, considerable interaction can occur between these vibrations within a single molecule (170) Multiple peaks thus appear in this region in the spectrum of rhodanine derivatives.

The imino group produces an absorption spectrum in the region of 3400 cm^{-1} to 3100 cm^{-1}(169). However, overlapping may occur at the frequency related to N-H and O-H stretching, so that an unequivocal differentiation in structure is sometimes impossible. Forfunataly the more stable structure of rhodanine is the one with a keto group (rather than a hydroxy group) at the 4-position, which obviates the confusion that may arise due to such overlapping.

In the aromatic moiety, ring stretching occurs in the general region between 1600 cm^{-1} and 1300 cm^{-1} The absorption involves stretching and contraction of all the bonds in the ring as well as the interaction modes. The bond patterns and the relative intensities depend on the substitution pattern and the nature of the substituents. Some characteristic bands of the functional group are shown below :

Functional group	Absorption region (cm^{-1})
Benzenoid ring	1600 - 1500
C = C	1700 - 1600
C - Cl	1096 - 1089
OH(free)	3650 - 3584
(H-bond)	3550 - 3200

The pyridine ring shows 4 bands in the region, closely resembling a monosubstituted benzene and thiophene ring displaying 2-4 bands (170).

In the case of tetra-acetyl-glucosides of rhodanine, there are no characteristic bands for any particular sugar. In order to identify compounds of this group, the spectrum should be observed over a wide range, usually between 4000 - 650 cm^{-1}, with the region between 1250 - 650 cm^{-1} being the most characteristic (171).

It is most important when measuring the spectrum of an unknown sample to ensure that it is in the same physical state as the compound used for comparison. The spectrum may differ considerably depending on the physical state of the compounds. In point of fact, shifts in band frequencies of up to 20 cm^{-1} in a spectrum for the same substance examined first in a crystalline and then in an amorphous state (as in mulling) does not usually destroy the crystallinity of the sample : but the preparation of a pressed disc may render a crystalline solid amorphous and this change is usually associated with a broadening of the absorption bands in the spectrum.

There are two important limitations to the use of infrared spectroscopy for identification of a carbohydrate (171) :

1. The difference between the spectra of consecutive members of a polymer series becomes very small after the first few units.

2. The spectra of D-and L-enantiomorphs of sugars are identical if they occur in the same crystalline form.

Fortunately, the compounds we are concerned with here are not polymers, since we are dealing only with the D-form. The problem under consideration is the possibility of producing an α - or β-anomer. Since the spectra of D and L enantiomorphs are identical, the assignments of the α , or β anomeric configugation is independent of whether the sugar is D or L. The charac-

teristic peaks for α - or β-forms, associated with the anomer C-H bonds, are between 855 - 833 cm^{-1} for the α-form and 905 - 876 cm^{-1} for the β-form (171) The tetra-acetyl glucoside derivatives obtained from this investigation showed an absorption band between 910 - 885 cm^{-1}, confirming that they are in fact β-form.

As regards the poly - O - acetyl group, the carbonyl stretching vibration of saturated aliphatic ester generally occurs at a higher frequency than that of normal ketone, and the band range is in the region of 1750 - 1735 cm^{-1} (170). The spectra of all tetra-acetyl glucosides examined in this study showed peaks within this region.

The last feature to be discussed is the acetyl-free glucoside moiety. Deacetylation of tetraacetyl glucopyranosyl derivatives theoretically produces a poly-hydroxy group. In general, the unbonded or free hydroxy group absorbs strongly in the region between 3650 - 3584 cm^{-1} (170). Sharp, "free" hydroxy bands are observed only in the vapor phase or in very dilute solution in nonpolar solvents. Intermolecular hydrogen bonding shows broader bands at lower frequencies 3550 - 3200 cm^{-1}. Intra-hydrogen bonding, which is essentially independent of concentration, causes a slight shift of absorption to long wavelength (3600 - 3436 cm^{-1}) as compared to free hydroxy absorption - and the band is generally sharp, though it may undergo a slight broadening. The spectrum of the compound concerned shows a broad

absorption band between 3500 - 3300 cm^{-1}

Several compounds of the deacetylated glucosides were found to be hydrated (see Table III). The hydrogen bonding between water and the glucoside causes an increase of the melting points. This evidence can be found in the case N-β-D-glucopyranosyl -5-benzylidene rhodanine which was previously reported as an anhydrous having a m.p. of 104 - 110°C (54), but it was found by this investigation that it formed a hydrated glucoside and the m.p. was increased to 156 - 158°C.