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PREFACE

The chemistry of sucrose has always been a particularly challenging problem to carbohydrate chemists. Considering the fact that sucrose has long been available in large quantity in a high state of purity, until redently, the compound has been subjected to surprisingly little organic chemical investigation other than efforts to establish its structure. The efforts have established sucrose to be α-D-glucopyranosyl-D-fructofuranoside. Physical evidence (X-ray analysis) had previously indicated the configuration of the anomeric center in the fructose moiety to be β-D-. The purpose of this research was mainly to establish the course of partial p-toluenesulphonation of sucrose. The conclusions reached by previous workers on this subject were either speculative or unwarranted and left the impression that the selectivity of the p-toluenesulphonation reaction merited more accurate delineation. Also, it was hoped to complete an unequivocal, purely chemical proof of the structure of sucrose. These objectives were fulfilled and the purpose of this thesis is to describe and interpret the relevant experimental data.

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The author wishes to express his heart-felt gratitude to his research supervisor, Professor R.U. Lemieux, for his incessant interest in the problem, his valuable instructions and guidance and his patient determination to develop in the author a true scientific attitude for research.

The author wishes finally to express his thanks to all the Professors and all members of the staff in the Faculty of Science of the University of Ottawa for their pleasant cooperation and the many helpful discussions held during these investigations.

ABSTRACT

The esterification of one mole of sucrose with three moles of p-toluenesulphonyl (tosyl) chloride in pyridine at 0° was reported (1,2), prior to these investigations to produce 1',6,6'-tri-O-tosylsucrose in "high yields". However, the compound was not obtained in a crystalline condition. The results of a recent attempt (3) to characterize the so-called "tritosylsucrose" threw little light on the subject. It has now been observed by chromatographic analysis using silicic acid, that the so-called "tritosylsucrose" contains penta-, tetra-, tri-, and di-O-tosylsucroses in the molar ratios 0.05:0.33:1:1, respectively. The composition of the product was substantially the same when the tosylation reaction was performed at -18°. The chromatogram separated the tri-O-tosylsucrose fraction into two sub-fractions. The major sub-fraction represented 29% by weight of the original "tritosylsucrose" and consumed three moles of oxidant per mole when treated with sodium metaperiodate. This required the material to be mainly 1',6,6'-tri-O-tosylsucrose. The minor sub-fraction represented 13% by weight of the original "tritosylsucrose" and consumed only two moles of oxidant. This required that at least one of the three

tosyloxy-groups be situated on a secondary carbon atom. Crystalline 6,6'-di-O-tosylsucrose was obtained from the di-O-tosylsucrose fraction and represented 10% by weight of the original "tritosylsucrose". A similar chromatographic analysis of the product from the ditosylation of sucrose has shown the product to contain tetra-, tri-, and di-O-tosylsucroses in the molar ratios 0.18:0.82:1, respectively. Crystalline 6,6'-di-O-tosylsucrose was obtained in a yield 20% by weight of the original "ditosylsucrose". The absence of mono-O-tosylsucrose in any of the above products may be due to losses during the isolation procedures.

The alkaline alcoholysis of 1',6,6'- tri-O-tosylsucrose was shown to produce 1',2;3,6;3',6'-trianhydrosucrose in high yield. 1',4,6'-tri-O-tosylsucrose pentaacetate was prepared by treatment of 2,2',3,3',6-penta-O-acetylsucrose (4) with tosyl chloride in pyridine at 0°. Alkaline alcoholysis of the compound was shown to produce 3,6-anhydro-α-D-galactopyranosyl-1,4;3,6-dianhydro-β-D-fructoside in high yield. This afforded unequivocal chemical evidence for the "β" configuration at the anomeric center of the fructose moiety of sucrose and thereby completed the proof of structure of sucrose by purely chemical means.

Examination of the product obtained from the tosylation of sucrose in the presence of aqueous sodium

hydroxide (Schotten-Bauman reaction) has shown the product to consist almost entirely of highly esterified sucrose.

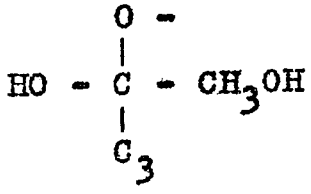
INTRODUCTION

1 - Sucrose, (α-D-Glucopyranosyl-β-D-Fructoside)

Sucrose, commonly known as table sugar, is by far the most abundant and most widespread carbohydrate found in the sap of plants. For many decades, sucrose from the sugar cane and, more recently, from the sugar beet has been the outstanding example of a very cheap, highly crystalline organic compound produced in a state of unexcelled purity on a large scale (above forty million tons in 1954). The compound, which is used almost exclusively as food, has engaged the minds of many chemists and biochemists through the years. However, besides efforts relating to the isolation and purification, the proof of its structure and synthesis, the compound has been the subject of relatively few published organic chemical investigations.

Sucrose is a sweet-tasting, highly crystalline solid which melts at 188° and has a specific rotation $[\alpha]_D$ + 66.53°. Hydrolysis of sucrose in the presence of acids or of specific enzymes yields equimolar quantities of D-glucose and D-fructose. Sucrose reduces Fehling's solution only after hydrolysis of the glycosidic linkage. Endeavors to elucidate the structure and configuration of

sucrose up to 1949 were reviewed by Levi and Purves (5). The results show that the D-glucose and the D-fructose of sucrose are linked through the anomeric carbon atoms and that a six-membered "pyranose" ring is present in the D-glucose portion and a five-membered "furanose" ring is present in the D-fructose portion. The specificity of the action of the enzyme "invertase", a β -D-fructofuranoside, which cleaves the sucrose molecule between the anomeric oxygen and the anomeric carbon of the D-fructose moiety (6), provided evidence for the α -configuration of the D-glucose moiety of sucrose. Since α-D-glucopyranose is the initial product, the -configuration for the D-glucose portion of sucrose is indicated (6,7). The argument for the β -D-configuration of the D-fructose portion of sucrose based on biochemical evidence was obtained (8) by showing that the enzyme "invertase" catalyses the hydrolysis of the less dextrorotary of the two fructofuranosides. Therefore, on the basis of Hudson's rules of isorotation, the anomers susceptible to invertase action were designated the β -anomers. However, there existed no evidence to support the idea that configuration of the anomeric center in a β -D-fructofuranoside is as follows,



Therefore, the only acceptable evidence for the β-D-configuration at the D-fructose portion of sucrose was that provided by X-ray crystallographic analysis (9), the results of which confirmed the other points of structure in the sucrose molecule. The first purely chemical evidence for the α-glucosidic linkage in sucrose was presented by Lemieux and Huber as a conclusion of the method used to synthesize sucrose (10). They synthesized maltose (11), trehalose (12), and isomaltose (13) using Brigl's anhydride. The fact that the latter three disaccharides are definitely α-D-glucopyranosides together with a rationalization of the properties of Brigl's anhydride could therefore be offered as synthetic evidence for the α-D-configuration of the glucosidic linkage in sucrose (10).

Thus, both chemical and physical evidence exists for every point of structure in the sucrose molecule except for the configuration of the anomeric center in the fructose moiety. For this center, only the X-ray evidence can be considered convincing. In view of the statement (14) that this X-ray analysis was an approximate one only, supporting chemical evidence seemed desirable.

The following diagram for sucrose (Figure 1), represents the compound as α-D-glucopyranosyl β-D-fructo-

furanoside. The conformational features are those expected on the basis that the molecule will possess a minimum of non-bonded interactions and are to a large part supported by the X-ray analysis of the compound in the crystalline state.

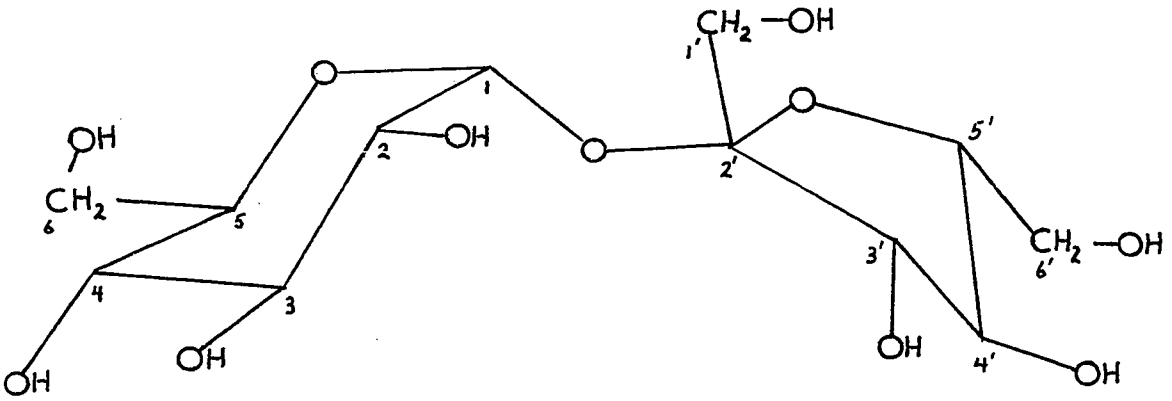


Figure 1. - Sucrose, α-D-glucopyranosyl β-D-fructofuranoside.

The system for numbering the positions of the sucrose molecule was proposed by Hockett and Zief (2) and consists in numbering the positions of the glucose portion with numerals and those of the fructose portion with prime numerals. An inspection of the sucrose molecule reveals the presence of three primary and five secondary hydroxyl groups. Although it is generally

accepted that primary hydroxyl groups are more reactive than secondary hydroxyl groups there exists no data on the relative reactivities of the various hydroxyl groups of sucrose.

2 - Preparation of Sulphonyl Esters of Carbohydrates

The use of sulphonyl esters of carbohydrates began as early as 1918 when Oden (15) discovered their usefulness for identification purposes via their physical properties, particularly since many of these esters crystallize with comparitive ease. However, various aspects of their chemical behaviour were soon accumulated and the remarkably interesting properties and potentialities of these compounds gradually came to be appreciated. Indeed, because of the kinds of reactions which they can undergo, sulphonyl esters have turned out to be one of the most useful groups of carbohydrate derivatives. p-Toluenesulphonyl (tosyl), methanesulphonyl (mesyl) and other organic sulphonyl esters have been prepared and are described in detail in an excellent review by Tipson (16). The tosyl esters, which have been particularly well studied, exhibit certain unique characteristics which make them of great importance in synthetic and analytical organic chemistry, especially in the field of carbohydrate chemistry. The preparation of

sulphonate esters is accomplished by treatment of a carbohydrate with a pyridine solution of an aryl or alkyl sulphonyl chloride (RSO_2Cl) or with 50% sodium hydroxide and the sulphonyl chloride at 0°. In the case of the partial tosylation of polyhydroxy compounds, the question arises as to whether there is, with respect to tosyl chloride, any variation in the difference in reactivity; (a) between primary and secondary hydroxyl groups, and (b) between two primary or two secondary hydroxyl groups. Compton (17) in 1938 obtained evidence that primary hydroxyl groups can undergo tosylation substantially more rapidly than do the secondary ones in a carbohydrate structure. He obtained yields of 41% and 36% of the 6-O-tosyl derivatives on mono-tosylation of the methyl α - and β -D-glucopyranosides, respectively. These results indicate a difference in reactivity between the primary positions and the average secondary positions in the order of 7. In another example, Compton (18) reports that the dimolar tosylation of methyl β -D-cellobioside in pyridine followed by acetylation resulted in the isolation of a 67% yield of crystalline methyl 6,6'-di-O-tosyl penta-O-acetyl-β-D-cellobioside. This indicates a difference in reactivity between the primary and the secondary positions of the order observed with trityl chloride (triphenylchloromethane) (19), a compound well recognised as highly specific towards primary hydroxyl

groups. Indeed Hockett and Downing (20) found that the secondary hydroxyl group in 1,2;5,6-di-O-isopropylidene-α-D-glucose and the primary group in 1,2;3,4-di-O-isopropylidene-α-D-galactose differed in reactivity by a factor of 70 and thus concluded that "the selectivity of the p-toluenesulphonyl chloride toward primary hydroxyl groups as compared with secondary ones is of the same order as the selectivity of triphenylchloromethane". It is of interest to note that the primary hydroxyl group in 2,3;4,6-di-O-isopropylidene-L-sorbose, which is similar to the 1'-hydroxyl group in sucrose, underwent tosylation half as rapidly as the primary hydroxyl group of the 1,2;3,4-di-O-isopropylidene-D-galactose (20). The above examples clearly indicate the widely varying receptivity of various primary and secondary hydroxyl groups towards acylation with tosyl chloride in pyridine. Little is known about the factors responsible for such a difference in reactivity. Undoubtedly, non-bonded interactions in the transition state are important and probably mainly responsible for the generally greater reactivity of primary positions (21). Figure 2 illustrates the probable steric relationship (22) of the reacting molecule in the transition state. It is readily recognised that replacing one hydrogen atom with a carbon atom undoubtedly increases the importance

of the non-bonded interaction with the substituents on the sulfur atom, and hence could be responsible for a difference in reactivity in the order of 10 between a

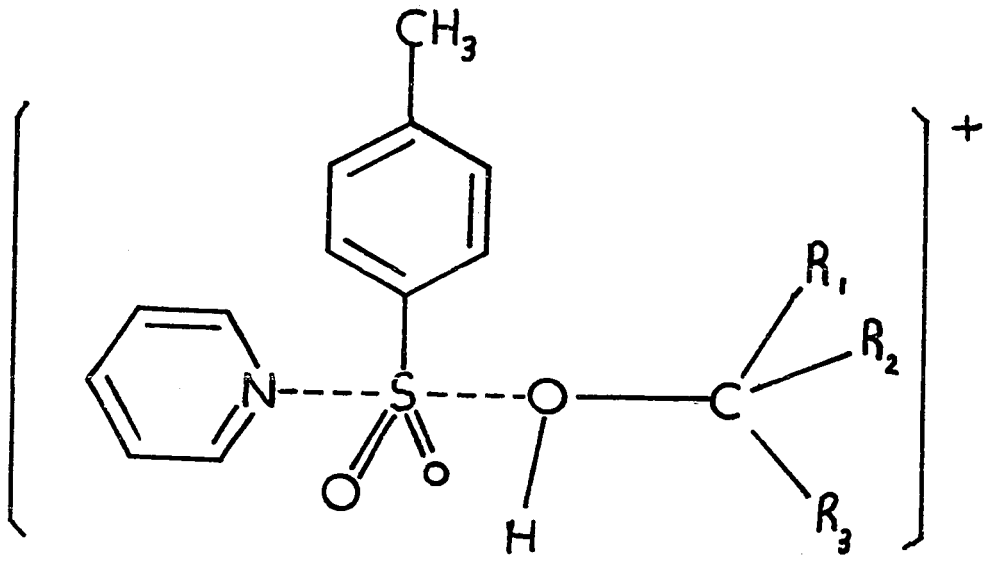


Figure 2. - A probable transition state of the tosylation reaction.

primary and a secondary hydroxyl group. Lemieux and McInnes (23) have recently shown that intramolecular hydrogen bonding also can strongly influence the rate of tosylation of secondary hydroxyl groups. Furthermore, little information is available on the relative rates for the tosylation of the starting material and the first product of tosylation. Experience in related fields has shown (24,25,26) that this can be a dominant feature

of the esterification of polyhydroxy compounds with hydrophobic reagents. Thus when it is considered that reactivities of both primary and secondary hydroxyl groups must vary considerably amongst themselves, it seems clear that attempts at preferential tosylation of the primary positions of polyhydroxy compounds can be expected to proceed in widely varying yields.

Raymond and Schroeder (1) in 1944, then Hockett and Zief (2) in 1950, reported the preparation of a substance termed "tritosylsucrose" by the treatment of one mole of sucrose with three moles of tosyl chloride in pyridine at 0°. The product obtained was not well characterized, nevertheless, it was suggested (2) that the product was probably "almost entirely" substituted at the three primary positions. The product gave a substance containing on the whole two iodine atoms per tosyl group, on treatment with sodium iodide in acetone at 100°. It was postulated that the 1'-tosyloxy group was not replaceable by iodine under these conditions. Since no direct evidence was available regarding the nature of this third tosyloxy group of the so-called "tritosylsucrose" and since the relative rates of tosylation of primary versus secondary hydroxyl groups remained obscure, the above contentions that the product was mostly composed of 1,6,6'-tri-O-tosyl-

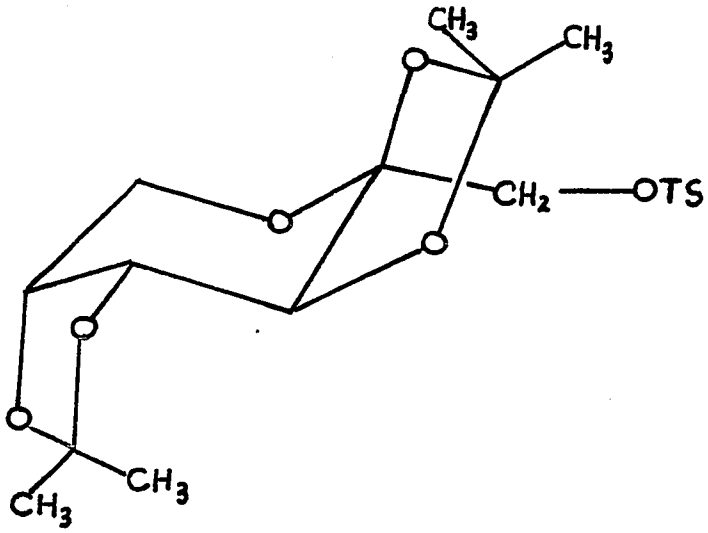
sucrose was doubtful and definitely required further investigation. Bragg and Jones (3) have recently made a study of this so-called "tritosylsucrose" by methylation followed by reductive detosylation, hydrolysis, and inspection of the partially methylated D-glucose and D-fructose derivatives thus formed. Inspection of their results reveals no basis for the contention that the substance "consisted mainly therefore of 1',6,6'-tri-O-tosylsucrose". Considerable doubt was entertained as to this characterization, first of all in view of ordinary kinetic consideration since it was simply assumed that the substance was an homogeneous tri-substituted sucrose derivative without ever considering the possibility of the presence of important amounts of mono-, di-, tri- and possibly other poly-substitution products. In this connection, the isolation of a dimethyl D-glucose derivative was rationalized by suggesting that the methylation was not complete. Finally, the yields of methylated D-glucose and D-fructose isolated were too low to permit the sweeping contention that the tritosylsucrose "consisted mainly therefore of 1',6,6'-tri-O-tosylsucrose". In view of these irregularities encountered with the so-called "tritosylsucrose" it seemed desirable to attempt a chromatographic separation of its constituents before any attempt at characterization was made.

Furthermore, it was felt that the many versatile reactions of the tosyloxy groups could be used to characterize the various tosyl esters of sucrose present in the mixture, and perhaps, secure further chemical evidence for certain points of structure of the sucrose molecule.

3 - Reactions of Sulphonyl Esters of Carbohydrates

The sulphonyl esters readily undergo carbon to oxygen cleavage. This property is not surprising in view of the fact that the departing group, a sulphonate ion, is the anion of a strong acid. The carbon to oxygen cleavage can occur either by solvolysis or by bimolecular displacement. The solvolysis of even the sulphonate esters of aliphatic alcohols proceed slowly. Thus this reaction route can be expected to be extremely unfavorable if the α-carbon (or carbons) is oxygenated as is usually the case in a carbohydrate structure. For example, Winstein and Buckles (27) have shown that cyclohexyl tosylate solvolyses in acetic acid 4.5×10^4 times more rapidly than cis-2-acetoxy-cyclohexyl tosylate. Thus, the solvolysis (S_N1) mechanism can be expected to be an unimportant route of reaction for sugar sulphonates unless there is a possibility for highly effective neighboring group participation such as can be provided by

a suitably situated acetoxy (28), acetamide (29), benzamide (30), or ethylthio group (31). In fact all of the reactions of sulphonate esters which proceed readily without intramolecular participation take place in the presence of reagents which are well recognised as strongly nucleophilic. Examples of these are the iodide ion (32), bromide ion (33), ammonia (34), hydrazine (35), and the thioethoxide ion (36). In every case where stereochemical results of such replacements could be established, inversion of the reacting center was observed as required for an S_N2 displacement (37). In accordance with this conclusion is the fact that, in general, sulphonyloxy groups at primary carbons are more easily replaced than those at secondary positions. It is a well established fact that the S_N2 process is particularly sensitive to steric inhibition (38). In accordance, even a primary sulphonyl ester can be highly hindered if the β-carbon is substituted by three large groups or atoms as in the neopentyl radical. Thus, it is not surprising that the 1-O-tosylate of 2,3;4,5-di-O-isopropylidene-D-fructopyranose is extremely resistant to attack by iodide ion (39).



1-O-Tosylate of 2,3;4,5-di-O-isopropylidene-D-fructopyranose.

Reaction, however, with sodium thioethoxide in dimethylformamide at 100° for 30 hours did bring about replacement of the tosyloxy group by the ethylthio group (40). Thus one could predict that a tosyloxy group at the 1'-position of sucrose would be resistant to replacement by iodine ion as compared to the 6 and 6' positions. Although the relative importance of steric, polar and inductive effects in determining reactivity has not well been established for the replacement of a tosyloxy group of a carbohydrate structure, it is clear from an abundance of published reactions that secondary tosyloxy groups are much less

readily replaced, as a rule, than are the primary tosyloxy groups. In fact, the reaction of a tosylated position of a carbohydrate with sodium iodide in acetone is widely used to test whether the position is primary or secondary (41).

Sulphonyl esters can undergo sulphur to oxygen cleavage (ester hydrolysis or alcoholysis) in the presence of strong bases such as the hydroxide or alkoxide ion. Thus, for example, boiling a 5% solution of 1,2;5,6-di-O-isopropylidene3-O-tosyl-D-glucose in 2.5 N potassium hydroxide (in 50% aqueous ethanol) for seven hours produced an "almost quantitative" yield of 1,2;5,6-di-O-isopropylidene-D-glucose (42). The yield of the diacetone glucose is surprising since an elimination reaction involving the 4-hydrogen which is <u>trans</u> to the tosyloxy group can be expected to take place to some extent.

Although alkoxide ions are generally of little use as nucleophilic reagents for the direct replacement of a sulphonyloxy group when used as external reagent, they can, however, be highly effective as internal reagents for intramolecular displacements. This is evident from the reactions of partially tosylated polyhydroxy compounds in the presence of strong base wherein an oxygen anion of a free hydroxyl group can make a nucleophilic attack at the tosylated center to displace the ester function to form an epoxide ring. The

most favorable situation for the formation of 2-epoxide is that which has the oxygen anion and the tosyloxy group in a true trans-relationship. That is, the configuration which has the two hydroxyl groups on opposite sides and in the same plane as the bridging carbon atoms (43). Thus, the oxygen anion, the reacting carbon and the departing group can readily achieve the linear arrangement required in the transition state for a displacement (S_N2) reaction. The formation of large epoxide rings, also called anhydro rings, requires the molecule to achieve in the transition state a conformation which has the attacking oxygen anion in close proximity to the side of the reacting carbon opposite to that occupied by the leaving group. Thus, the reactions shown in Fig. 3 p. 19 must proceed by way of conformations at least approaching those illustrated. There exists no evidence that the energy barrier for these conformation changes can be nearly as great as that for the chemical reaction. In fact, as far as can be gathered from the available information, all such chair-chair and chair-boat interconversions are extremely rapid and, therefore, represent only trivial intermediates in the achievement of the required transition states. These reactions were chosen to illustrate the formation of the kinds of ring systems (similar to the bicyclo (3.2.1) octane, bicyclo (2.2.1) heptane, bicyclo (4.1.0) heptane and bicyclo (4.4.0) decane ring systems)

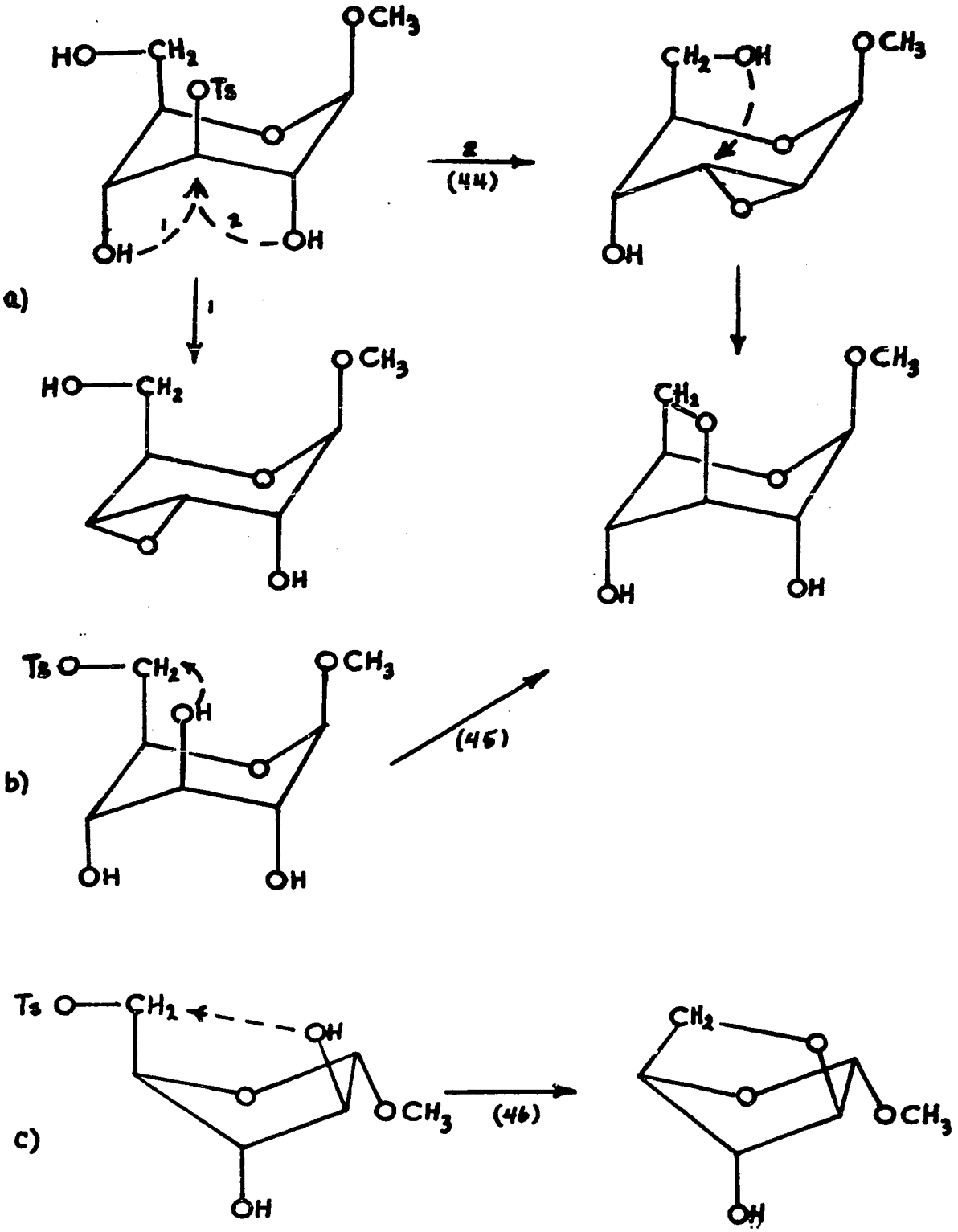


Figure 3. - Examples of Epoxide Formation.

encountered in the present research.

Although the α- or three membered epoxides are formed more readily than the larger epoxy or "anhydro" rings, when the possible conformations of the molecule can satisfy the requirements for the S_N2 transition state for both cases, the former are, subsequently, more susceptible to further anionic attack. Consequently, an α-epoxy sugar may be the initial product of alkaline elimination of an halogen or sulphonic ester, but may in turn undergo a nucleophilic attack by a suitably situated oxygen anion. Thus, the α-epoxy sugar may undergo intramolecular rearrangement to another α-epoxy sugar or to a more stable "anhydro" type epoxide (fig. 3a). This type of intramolecular rearrangement is termed "epoxide migration" and further examples of such reactions are illustrated in Figure 4 (p. 21).

"Epoxide migration" was first postulated ty Lake and Peat (47) to explain the formation of two epoxides, instead of the expected one on treatment of methyl 2-O-tosyl-β-D-glucopyranoside with sodium methoxide, figure 4a, the methyl 2,3-anhydro-β-D-mannopyranoside and methyl 3,4-anhydro-β-D-altropyranoside. Buchanan (48) recorded another, similar case. Newth (49) also assumed the occurence of epoxide migration when he found that 1,6-anhydro-β-D-altropyranose gave, not the expected 1,6;2,3-dianhydro-β-D-mannopyranose

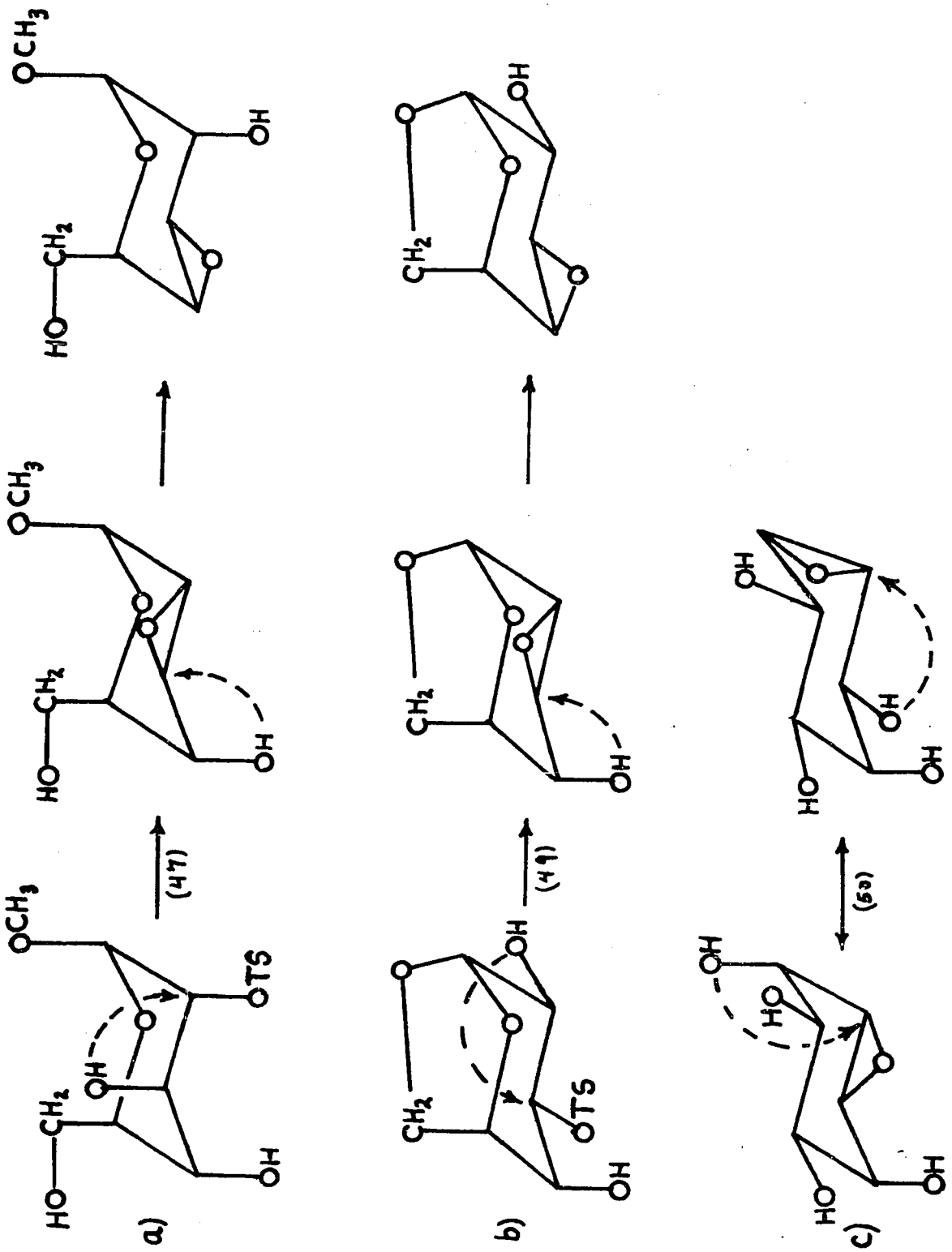


Figure 4. - Epoxide migration.

but the 1,6;3,4-dianhydro- β -D-altropyranose (fig. 4b). Angyal and Gilham (50) have definitely established the occurence of epoxide migration in the cyclitol series by converting one α -epoxide into another which was itself isolated, and by studying the position of the equilibrium of the two epoxides (fig. 4c). The opening of the α - epoxide is a nucleophilic displacement on carbon occuring with inversion, therefore, the entering group must attack the carbon from the side opposite to that of the epoxide-oxygen. Since after migration, the new hydroxyl group will be trans- situated in respect to the new epoxide it will be in a suitable position for nucleophilic attack to reverse the reaction. Should the reaction be in fact reversible then the epoxide ring migration will tend to the equilibrium mixture of the two epoxides (50). Of course, the true equilibrium may not be achieved since one or both of the oxides may enter into competing and irreversible reactions with either external nucleophilic reagents or internally with some suitably situated hydroxyl group.

EXPERIMENTAL

All melting points are uncorrected and were determined with a Leitz, hot-stage apparatus. The rotations were measured at room temperature, 20-25°, using the D-line of sodium. Infrared spectra were obtained with a Perkin-Elmer, single-beam, double-pass instrument. Optical densities for colorimetric determinations were obtained from a Coleman-Junior-Spectrophotometer. Micro-analyses for carbon and hydrogen were carried out in part by Geller Laboratories, West Englewood, N.J. and in part by Miss E. Buske, Department of Chemistry, University of Ottawa.

The chemical reagents used in the experimental part were of reagent grade unless otherwise specified.

1 - A Chromatographic Analysis of Sucrose Tritosylate and Ditosylate "Tritosylsucrose"

A preparation was made at 0° in 95% yield using the method described by Hockett and Zief (2), and the product had a m.p. of 65-85°, $[\alpha]_D = +43°$ (c, 2.88 in chloroform). Anal. Calc. for $C_{33}H_{40}O_{17}S_3$: S, 11.93%. Found: S, 11.11%. The composition of this product is described in Table I.

A second preparation was made at -18° using the following procedure. Sucrose, 34.2 g (0.1 mole), was dissolved

dry pyridine, 600 ml, by refluxing the mixture for a short period of time. The solution was then cooled with vigorous stirring to -18° for two hours using a refrigerated bath. Tosyl chloride, 57 g (0.3 mole), was dissolved in 150 ml of dry pyridine and the solution was added dropwise to the sucrose solution over a period of two hours. The reaction was left with continuous stirring at -18° for five days. The product, isolated in the usual manner (2), weighed 74.3 g (92.5% yield), with m.p. 65-85°, $[\alpha]_D = +42°$ (c, 2.5 in chloroform). Anal. Calc. for $C_{33}H_{40}O_{17}S_3$: S, 11.93%: Found: S, 11.66%.

The Chromatography of Tosyl Esters of Sucrose on Paper Impregnated with Silicic Acid

The paper was prepared using the method described by Marinetti, Erbland and Kocken (48), except that Whatman No. 3 paper was used. Silicic acid, 300 g was dissolved in one liter of 7.2 N sodium hydroxide. The solution was then made up to 1.7 liter. Strips of paper (4 in. wide) were immersed in the solution and hung over the tray which contained the sodium silicate solution. When the excess solution had dripped off the paper, it was immersed in 6 N hydrochloric acid, cooled to 4°, kept in a tray for 30 minutes, then washed in running water, and, finally, in distilled water.

The paper strips were dried and the adsorption capacity of the silicic acid was regenerated by heating the paper strips at 120° for one hour.

The samples were applied to the paper in the usual manner. The chromatograms are developed using the "descending" method with the solvent system, diisobutylketone; acetic acid; water (40:25:5) (51). Ten to twelve hours were necessary for the solvent front to advance 40-50 cms. The developed paper strips were dried and the tosylsucroses detected by immersing the paper strips in 0.01% aqueous solution of Rhodamine 6G (51). The wet paper strips were inspected in a dark room under ultraviolet light, where the various tosylsucroses appeared as colored fluorescent spots on a yellow background.

Both "tritosylsucroses" prepared at 0° and at -18° produced spots at R_f values 0.06 (faint), 0.14, 0.38, 0.6, and 0.85.

The Silicic Acid Column Chromatography of "Tritosylsucrose"

The silicic acid, 200 g, was packed as a slurry in chloroform in a 2.5 cm diameter column. The "tritosylsucrose", 9-10 g, was dissolved in 20 ml of chloroform and applied to the top of the column. The column was then developed with the same solvent system used for the chromatograms on paper impregnated with silicic acid. When the new solvent had

displaced all the chloroform from the column, 3 ml fractions were collected at a rate of one tube every two minutes. A total of 110 tubes were collected. Further eluent was free of non-volatile material. The tubes were examined by spotting on paper (Whatman No. 1) and dipping the paper in aqueous Rhodamine 6G solution. Five different zones were apparent in the series of tubes. After combining the tubes within each zones, the solvent was removed in "vacuo" and the weights of the residues determined. The results are given in Tables I(Page 30) and II (Page 31). Crystals were deposited in fraction D which proved to correspond to a fraction of the di-O-tosylsucroses. In the case of the tritosylate prepared at 0°, the crystalline substance was collected before evaporation of the solvent. The extremely fine needles, 0.92 g, m.p. 101-106°, were extremely soluble in methanol but sparingly soluble in water, ethanol or chloroform. After three recrystallizations from water, the compound melted at 108-110°, $[\alpha]_D$ +54° (c, 1.60 in ethanol). The compound only reduced Fehling's solution after acid hydrolysis. Anal. Calc. for $C_{26}H_{34}O_{15}S_2$: C, 47.8%; H, 5.22%; S, 9.85%. Found: C, 47.2%; H, 5.29%; S, 10.1%. The experiments described below established the compound to be 6,6'-di-O-tosylsucrose.

6,6'-Dideoxy-6,6'-diiodosucrose Hexaacetate

6,6'-Di-O-tosylsucrose, 0.1 g, was dissolved in one ml of dry pyridine and two ml of acetic anhydride and left at 4° for two days. The acetate product, 126 mg, was isolated in the usual manner. The amorphous solid has resisted crystallization thus far. The substance, 76.5 mg, was dissolved in 1.5 ml of 10% sodium iodide in acetone and the solution was heated at 120° for 16 hours. The sodium tosylate, 36.2 mg, which precipitated, represented a replacement of two tosyloxy groups. The syrupy product, 59.2 mg, was isolated in the usual manner. Its nuclear magnetic resonance spectrum was devoid of signals for the aromatic and C-methyl hydrogen atoms of the tosyl group. Anal. Calc. for $C_{24}H_{32}O_{15}I_2$: I, 31.4%. Found: I, 29.3%.

Monoiodomonodeoxy-O-tosylsucrose Hexaacetate

The syrupy di-O-tosylsucrose (fraction D), 2.12 g, remaining after removal of the crystalline 6,6'-di-O-tosylsucrose (see above), was acetylated and the product was subjected to the sodium iodide in acetone treatment. The amount of sodium tosylate recovered represented a replacement of 1.1 tosyloxy group.

The Reaction of Tri-O-tosylsucrose A and B with Sodium Iodide

Acetylated samples of tri-O-tosylsucrose fractions "A", 135 mg, and "B", 128 mg, were treated as described above with 10% sodium iodide in acetone. The amounts of sodium tosylate recovered were 49.7 mg, and 41.6 mg, respectively. These amounts represent the replacement of 1.87 and 1.63 tosyloxy groups per mole, respectively. Anal. Calc. for $C_{29}H_{36}O_{16}I_2S$: I, 30.7%. Found: I, 28.6% (fraction "A") and 27.8% (fraction "B").

The Periodate Oxidation of the Tri-O-tosylsucrose "A" and "B"

To a sample of the ester, 0.05 millimole, dissolved in 80 ml of glacial acetic acid, 10 ml of 0.25 N sodium metaperiodate was added and the solution was made up to 100 ml with water. Aliquots, 10 ml were taken at suitable time intervals and reacted with 5 ml of 10% potassium iodide in a stoppered Erlenmeyer flask and left aside for one minute. Sodium thiosulphate, 10 ml of 0.1 N, was pipetted into the flask and the excess thiosulphate was determined by titration to the starch end point with standard 0.005 N iodine solution. Periodate was not consumed in the blank determinations. The periodate uptake by fraction "A" was 0.61, 1.53, 2.02, 2.18, 2.41, 2.78, and 2.82 moles of oxidant per mole of "A" after 0.5, 1.0, 10, 18, 32, 85, and 108 hours. The periodate

uptake by "B" was 0.49, 0.51, 0.98, 1.18, 1.59, 1.72 , and 1.75 moles of oxidant per mole of "B" after 0.5, 1, 10, 18, 32, 85 and 108 hours.

The Ditosylation of Sucrose

Sucrose, 34.2 g (0.1 mole), was dissolved in 600 ml of dry pyridine and cooled to 0°. Tosyl chloride, 38 g (0.3 mole), was added and the reaction mixture was kept at 0° for five days. The reaction mixture was then shaken at room temperature with 5 ml of water for 30 minutes. The excess pyridine was removed in vacuo at 40° to leave a thick syrup. The syrup was dissolved in one liter of chloroform and washed with 200 ml of ice-cold 2 N sulphuric acid followed with 200 ml of water. The chloroform solution, after drying over anhydrous sodium sulphate, was evaporated to a dry amorphous solid, 56 g (86% of theory).

Chromatography on silicic acid-impregnated paper revealed three components of R_f values; 0.13, 0.38, and 0.60. Column chromatography on silicic acid of ten grams of the material allowed the isolation of the fractions described in Table III, page 32.

TABLE I

Chromatography of "Sucrose Tritosylate"

The material, 9.05 g, was prepared at 0°.

	Penta-O-tosyl-sucrose	Tetra-O-tosyl-sucrose	Tri-O-tosylsucrose Fraction "A"	Tri-O-tosylsucrose Fraction "B"	Di-O-tosylsucrose Fraction "C"[1]	Di-O-tosylsucrose Fraction "D"
Color of fluorescence	red	pink	yellow	pink	yellow	yellow
Tube No.	17-22	24-34	39-57	58-62	93-105	86-110
Weight of material (g)	0.18	1.53	2.65	1.17	0.92	2.11
Percent of mixture (w/w)	2.0	17.0	29.0	13.0	10.0	24.0
Sulphur content (%) found:	13.9	13.40	11.84	11.41	10.12	9.22
calc:	14.4	13.38	11.93	11.93	9.85	9.85
$[\alpha]_D$ (chloroform)	+42.2	+35.0	+37.5	+33.0	+54.0	+35.0

[1] Crystalline 6,6'-di-O-tosylsucrose.

TABLE II

Chromatography of "Sucrose Tritosylate"

The material, 10, was prepared at -18°.

	Penta- and Tetra-O-tosyl-sucrose	Tri-O-tosylsucrose		Di-O-tosylsucrose	
		Fraction A	Fraction B	Fraction C[1]	Fraction D
Weight of material (g)	2.0	3.11	1.5	0.75	2.85
Percent of mixture (w/w)	20.0	31.1	15.0	7.5	28.5
R_f value[2]	0.9 and 0.65	0.40	0.40	0.15	0.15

[1] Crystalline 6,6'-di-O-tosylsucrose.

[2] On paper impregnated with silicic acid using the solvent system diisobutyl ketone: acetic acid: water (40:25:5).

TABLE III

Chromatography of "Sucrose Ditosylate"

The material, 10, was prepared at 0°.

	Tetra-O-tosyl-sucrose	Tri-O-tosylsucrose Fraction A	Fraction B	Di-O-tosylsucrose Fraction C[1]	Fraction D
Weight of material (g)	1.04	2.79	1.74	1.85	2.60
Percent of mixture (w/w)	1.04	27.9	17.4	18.5	26.0
Sulphur content (%)					
Found:	12.96	11.82	11.76	10.12	10.11
Calc.:	13.38	11.93	11.93	9.85	9.85

1 Crystalline 6,6'-di-O-tosylsucrose.

Detosylation of 1',6,6'-tri-O-tosylsucrose - (FRACTION A) - 1',2:3,6:3',6'-trianhydrosucrose Diacetate

Chromatographed tri-O-tosylsucrose A (see Tables I and II), 200 mg, was dissolved in methanol N in sodium methoxide and the solution was refluxed for 30 minutes. The reaction mixture was evaporated to dryness and the residue was shaken with boiling pyridine, 50 ml, for 15 minutes. After the mixture was cooled to 0°, 10 ml of acetic anhydride was added. The syrupy product, isolated in the usual manner, crystallized on trituration with methanol. The yield was 71.6 mg (77.4% of theory) of 1',2:3,6:3',6'-trianhydrosucrose diacetate, m.p. 179-181°: $[\alpha]_D$ = +127.4° (c, 1.22 in chloroform). The structure of this compound was established as described on pp. 35 - 40.

Detosylation of Crude "tritosylsucrose" - The Formation of 1',2:3,6:3',6'-Trianhydrosucrose (II)

"Tritosylsucrose", prepared by the method of Hockett and Zief (2), 175 g, was dissolved in one liter of M sodium ethoxide in ethanol and the solution was refluxed for one hour. The residue obtained after evaporation of the ethanol in vacuo, was partitioned between 750 ml of water and 250 ml of chloroform. The aqueous layer was extracted again with 50 ml of chloroform which, after washing

with an equal volume of water, was combined with the main chloroform extract. Evaporation of the chloroform gave 22 g of dark-brown syrup, the composition of which has not been investigated further than establishing the presence of sulphur. The combined aqueous extracts were extracted continuously with ether for two weeks. Evaporation of the ether extracts left a residue which on crystallization from ethanol gave 1.78 g (3.5% overall yield) of crude trianhydrosucrose m.p. 158-160°. Two further recrystallizations from ethanol afforded the pure compound, 1',2:3,6:3',6-trianhydrosucrose (II), m.p. 163-164.5°, $[\alpha]_D$ +117° (c, 0.9 in water). Anal. Calc. for $C_{12}H_{16}O_8$: C, 50.00%; H, 59%. Found: C, 49.73%; H, 5.75%.

The diacetate of 1',2:3,6:3',6-trianhydrosucrose was prepared in the usual manner, using acetic anhydride and anhydrous sodium acetate. The compound, m.p. 181.5-182.5°, $[\alpha]_D$ +128.6° (c, 1.8 in chloroform) was isolated in the usual manner and purified by recrystallization from ethanol. The molecular weight was determined by the Rast method and was 362 g (theory = 372 g). Anal. Calc. for $C_{16}H_{20}O_{10}$: C, 51.61%; H, 5.41%; sapon. equiv., 186.2. Found; C, 51.53%; H, 5.34%; sapon. equiv., 185.

The Structure Elucidation of the Trianhydrosucrose (II) as 1',2:3,6:3',6'-Trianhydrosucrose

The acid hydrolysis of trianhydride II was performed in the following manner (see figure 8) page 64 . A sample, 0.053 g, was dissolved in 5 ml of 0.01 N hydrochloric acid at 25°. The change in rotation of this solution was observed at intervals and the rotation became constant after 8 hours. The final specific rotation of this solution, based on the concentration of trianhydride II at the start of the hydrolysis, was +87° (c, in 0.04 N hydrochloric acid). Sucrose in 0.04 N hydrochloric acid at 25° requires approximately 1600 hours (52) for 99.99% inversion. The solution was deionized by percolating through a small column of Dowex 1X8 anion exchange resin (quaternary ammonium type) and evaporated in vacuo to a syrup, 54.6 mg. Paper chromatography (n-butanol-water) of this syrupy product showed only one main component of R_f value = 0.36, when examined with aniline phthalate spray reagent (53). A faint streak was also present from the origin to R_f = 0.45. The material was chromatographed on a Celite 535 column, 25 g. in the manner described by Lemieux, Bishop and Pelletier (54). The main band gave 39.1 mg of material, $[\alpha]_D$ = +.87° (c, 0.69 in water), R_f = 0.36 (n-butanol-water), which reduced Fehling's solution. The chromatographically pure hydrolysate (III) has

thus far resisted crystallization.

Periodate oxidation of compound (III), in 0.025 N sodium metaperiodate at 0°, consumed 1.73, 1.85, 1.88, 1.90, 1,92, moles of oxidant per mole after 2, 10, 60, 120, and 720 minutes. Titration of the reaction mixture, after destroying the excess periodate with ethylene glycol, indicated the formation of one mole of acid per mole of hydrolysate (III) oxidized. However, no formic acid nor formaldehyde could be detected using the following method. A weighed sample of the compound, ca. 0.05 millimole, dissolved in 2 ml of 0.2 M sodium metaperiodate and left to oxidize for the required time. When the oxidation has progressed to the desired extent, the oxidation mixture is quantitatively transfered into the "micro vacuum distillation" apparatus described by Grant (55), and the solution is frozen in an acetone dry-ice mixture. The apparatus is then evacuated to less than 1 mm. The arm of the apparatus which contains the frozen reaction mixture is then removed from the freezing mixture and the other arm is kept in the freezing mixture until all the volatile components have sublimed to the cold portion of the apparatus. The distillate, after thawing, is quantitatively transferred to a 25 ml volumetric flask and aliquots are titrated with standard 0.01 N sodium hydroxide to estimate the total

amount of acid present in the distillate. The acid can be conveniently characterized by converting a suitable aliquot to sodium salt, 1-2 mg, and dissolving the salt in 4 ml of 10% aqueous potassium bromide, freeze-drying and pressing the residue into a window in the usual manner (56). The sodium salt of formic acid is identified by its infrared spectrum. The formaldehyde content of the distillate is also conveniently determined colorimetrically using the chromotropic acid reagent (57).

The above method gave the following results on known compounds.

	Formic acid moles/mole	Formaldehyde moles/mole
D-sorbitol	3.905	1.88
D-glucamine (1-amino sorbitol)	2.94	0.92
D-glucose diethyl mercaptal	2.81	0.92

A sodium borohydride reduction of compound (III) to compound(IV) (see figure 8 page 64), was performed in the following manner. The trianhydride II, 100 mg, was hydrolysed in 0.001 N hydrochloric acid to constant rotation in order to obtain a solution of compound (III). The solution was neutralized with 0.05 N sodium hydroxide and then treated with a twofold excess of sodium borohydride at 0° for 36 hours. The excess sodium borohydride was destroyed with acetic acid and the resulting solution was evaporated in vacuo to dryness. The dry residue was acetylated using

5 ml of acetic anhydride, and 5 ml of pyridine. The acetylated product, 92.4 mg, was isolated in the usual manner and deacetylated in dry methanol, 10 ml saturated at 0° with ammonia. Paper chromatography of the crude reduced product (IV), 60 mg, indicated a main component of R_f = 0.26 (butanol-water). There was also a faint streak from the origin to R_f = 0.28. This syrupy product was chromatographed on a 25 g celite column in the usual manner (54). The isolated product (IV) did not reduce Fehling's solution and failed to crystallize. Periodate oxidation of compound (IV) in 0.025 N sodium metaperiodate at 0° consumed 1.8, 1.85, 1.91, 1.95 and 1.96 moles of oxidant per mole after 2, 15, 30, 60, 120 and 720 minutes. No formic acid nor formaldehyde was produced in this oxidation. Periodate oxidation of erythritan (58) (1,4-anhydroerythritol) in 0.25 N sodium metaperiodate at 0° consumed 0.95, 0.97, 0.99, 0.99, 0.99, and 0.99 moles of oxidant per mole after 2, 5, 10, 30, 60 and 120 minutes. However, oxidation of threitan (58) (1,4-anhydrothreitol) in 0.025 N sodium metaperiodate at 0° consumed 0.135, 0.153, 0.168, 0.210, 0.434, 0.930 and 0.99 moles of oxidant after 2, 5, 15, 70 150, 660 and 1320 minutes.

A dihexitol ether (V) (see Figure V), was obtained in the following manner. The crude acetyl derivative of

compound (IV), 96 mg prepared as described above from 100 mg of the trianhydride II , was treated with acetic anhydride, 1 ml, and concentrated sulphuric acid, 0.08 ml, at 0° for three days. The resulting acetolysis product was isolated in the usual manner and deacetylated by percolating through a column of Dowex 1X8 anion exchange resin to yield 63.8 mg of syrupy polyol. This product was chromatographed on a 25 g Celite column (butanol-water) (54). The main band, R_f = 0.24, afforded 39.7 mg of crystalline dihexitol ether (V), m.p. 105-109°. After two recrystallizations from ethanol, the m.p. was 110-114° (dimorphic), $[\alpha]_D^{25}$ - 12.6° (c, 0.48 in water). The periodate oxidation of (V) produced 5 moles of formic acid and 2 moles of formaldehyde per mole, based on the assumption that the compound corresponds to structure (V) (see Figure 8, page 64).

2-(β-hydroxyethoxy)-1,3-propanediol, compound (VII), was obtained by the following procedure. The dihexitol ether (V), 20.2 mg, was dissolved in 2 ml of 0.11 M sodium periodate and left to oxidize to a final rotation of 0° presumably forming the intermediate compound VI (see Figure 8, page 64). Excess sodium borohydride, 250 mg, was added to reduce the oxidation product VI and the solution was left for 18 hours before acidification with acetic acid to destroy the excess sodium borohydride.

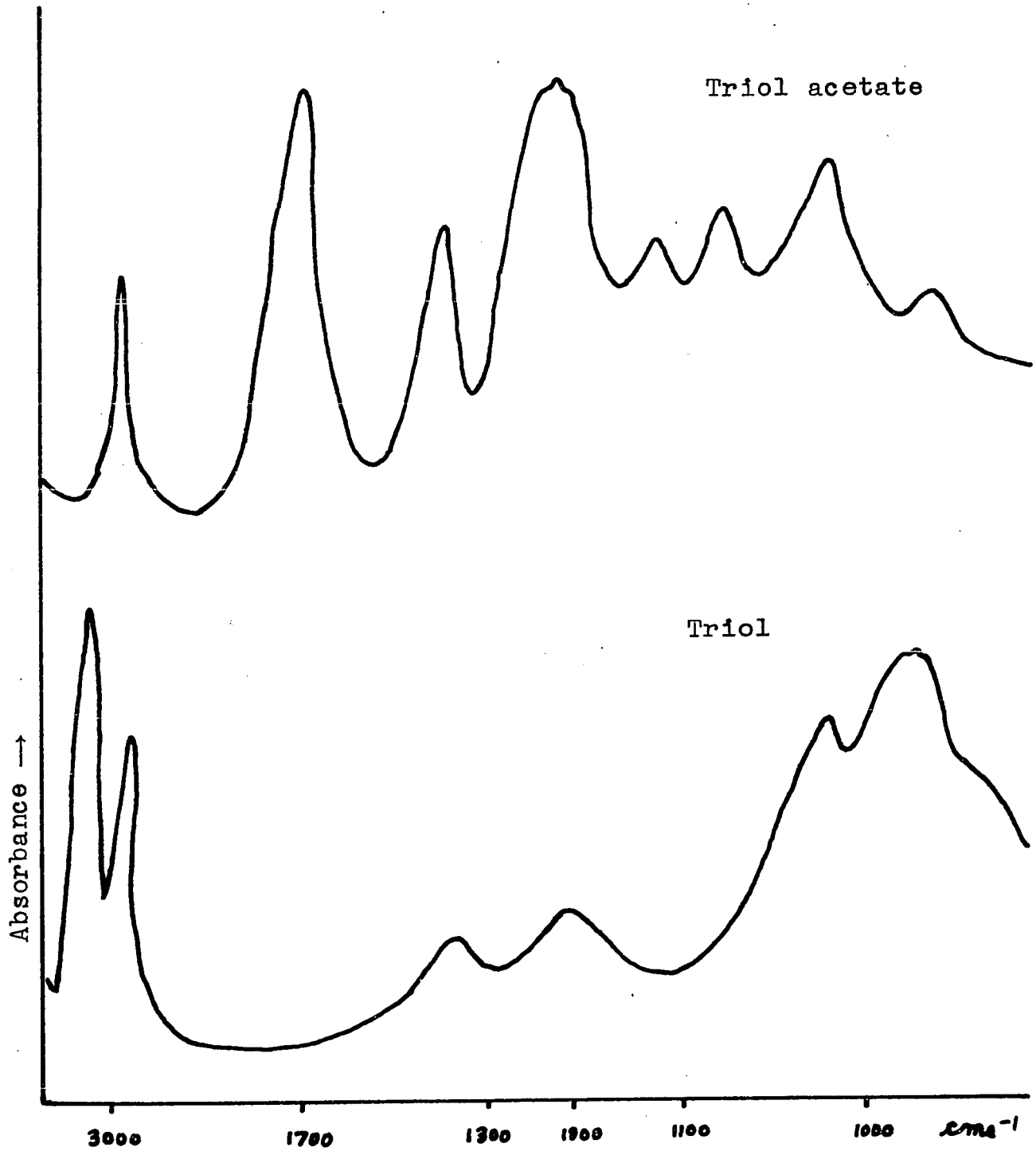


Figure 5. - Infrared Spectra of 2-(β-hydroxyethoxy)-1-3-propanediol and its acetate.

The resulting solution was evaporated in vacuo to a dry cake which was acetylated with acetic anhydride in pyridine in the usual manner to yield 12.1 mg of syrupy acetate. This syrupy acetate was distilled at a bath temperature of 125-135° and 0.3 mm pressure to yield 8.2 mg of yellowish syrup. The infrared spectrum of this syrupy acetate was almost identical with that of 2-(β-hydroxyethoxy)-1,3-propanediol triacetate prepared by an identical treatment of polygallitol (1,5-anhydrosorbitol). The syrupy triacetate was then deacetylated in absolute methanol, 3 ml, saturated with ammonia at 0°. The resulting syrupy triol (VII) was chromatographed on Celite in the usual manner (54). The main product, 2-(hydroxyethoxy)-1,5-propanediol (VII) had the same R_f, 0.48 (butanol-water), and identical infrared spectrum as the authentic specimen prepared in a similar manner from polygallitol (VIII) (see fig. 5). Reacetylation of the triol (VII) gave a product which gave a spectrum identical to that of the authentic triacetate prepared from polygallitol.

The Stability of 1',2:3,6:3',6'-Trianhydrosucrose in Bases

The trianhydride (II) remained unchanged when heated at 100° in 25% potassium hydroxide for 24 hours as

in 85% hydrazine at 100° for 36 hours. Similarly, the di-O-tosylate of (II), m.p. = 164-166°, when heated with 10% sodium iodide in acetone or with hydrazine in a sealed tube for 24 hours, remained unchanged.

4,4'-Di-O-Methyl-1,2':3,6:3',6'-Trianhydrosucrose

Trianhydride (II), 200 mg, was methylated by treatment with 10 ml of methyl iodide and 0.5 g of silver oxide. The mixture was refluxed with mechanical stirring. After 15 minutes another 0.5 g of silver oxide was added and this procedure was repeated until a total of 2 g of silver oxide was added. After refluxing overnight, the silver oxide was filtered and washed several times with boiling ethanol. The combined filtrates were evaporated and the residue was decolorized with charcoal. The compound crystallized after trituration with ethanol. After one recrystallization from ethanol, the yield was 147 mg of di-O-methyl trianhydrosucrose, m.p. 179-181°, $[\alpha]_D = +140°$ (c, 1.9 in chloroform). Anal. Calc. for $C_{14}H_{20}O_8$: methoxyl, 19.68%; Found: 19.78% and 20.05%.

The acetolysis of the 4,4'-di-O-methyl-1,2';3,6;3'6'-trianhydrosucrose, 66 mg, by treatment with acetic anhydride, 1 ml, and concentrated sulphuric acid, 0.03 ml, at 0° for 18 hours in the usual manner, gave 35.6 mg of syrupy product,

after deacetylation. Attempts to isolate either 4-methyl-D-glucose or 4-methyl-D-fructose from the product failed, probably because of the inability of the acetolyzing agent to cleave the 1,2 ether type linkage of this trianhydrosucrose molecule.

1',4,6'-Tri-O-Tosylsucrose Pentaacetate (IX)

2,2',3,3',6-penta-O-acetylsucrose, was prepared by the method described by McKeown, Serenius and Hayward (4). This compound, m.p. 155-156°, $[\alpha]_D$ +22° (c, 3.1 in chloroform), was treated with a twofold excess of tosyl chloride in dry pyridine for one week at 5°. After this time, the excess tosyl chloride was decomposed by the addition of water and the solution was poured as a fine stream into a large excess of cold water. The amorphous white precipitate was washed first with 2% hydrochloric acid and finally with water. The dry amorphous product (IX), m.p. 85-91°, was obtained in 91% yield and resisted crystallization. Anal. Calc. for $C_{43}H_{50}O_{22}S_3$: S, 9.45%; Found: S, 9.12%.

The sucrose tritosylate pentaacetate (IX), 400 mg, was heated at 100° for 17 hours with 1 g of sodium iodide in 25 ml of acetone. The amorphous product, 378 mg, (89% yield), isolated in the usual manner, possessed an iodine content, 12.92%, near that expected (12.81%) for monoiodomonodeoxysucrose ditosylate pentaacetate.

The Detosylation of 1',4,6'-Tri-O-Tosylsucrose Pentaacetate IX. - The Formation of 3,6-Anhydro-α-D-Galactosyl-1,4;3,6-Dianhydro-β-D-Fructoside (X)

The sucrose tritosylate pentaacetate (IX), 15 g, was added to 200 ml of N sodium ethoxide in ethanol. The solution was refluxed for two hours and the ethanol was removed by distillation under vacuum. Examination of the residue by paper chromatography revealed that the tritosylate (IX) was converted in high yield to a substance with R_f 0.36 (butanol-water). Only traces of two other products with higher R_f values could be detected. The residue was dissolved in water and the dark-brown solution was extracted with ethyl-methyl ketone continuously for one week. Evaporation of the solvent left 1.5 g of a residue which proved to contain sodium tosylate. The substance was consequently deionized by percolating an aqueous solution first through a bed of quaternary ammonium type resin (Dowex 1X10) and then through a bed of carboxylic acid type resin (Amberlite IRC-50-H). Evaporation of the water in vacuo left a crystalline residue which was recrystallized from ethanol. The yield of pure material (X), was 0.86 g. The infrared spectrum differed markedly from that of the trianhydrosucrose (II) both when determined as Nujol mulls and in potassium bromide windows. Anal. Calc. for $C_{12}H_{18}O_8$: C, 50.00%; H, 5.59%. Found: C, 50.00%; H, 5.71%.

The diacetate of trianhydrosucrose (X), m.p. 137.5-138.5°, $[\alpha]_D$ +94.3° (c, 2 in chloroform), was prepared by heating with acetic anhydride and sodium acetate in the usual manner. Anal. Calc. for $C_{16}H_{20}O_{10}$: C, 51.51%; H, 5.41%: Sapon. equiv., 186.2. Found: C, 51.42%: H, 5.50%: Sapon. equiv., 185.

The Di-O-Methyl Derivative of Compound X

The compound X, 200 mg., was added to 10 ml of methyl iodide containing 0.5 g of silver oxide. The mixture was heated under reflux with mechanical stirring. After 15 minutes a second 0.5 g of silver oxide was added and this procedure was continued until a total of 2 g of silver oxide was added. After refluxing overnight the silver oxide was collected by filtration and washed several times with boiling ethanol. The combined filtrates were evaporated and the residue was decolorized with charcoal and crystallized from ethanol. The yield was 150 mg., m.p. 105-106°, $[\alpha]_D$ +48.6 (c, 1.45 in chloroform), Anal. Calc. for $C_{14}H_{20}O_8$: methoxyl 19.62. Found: methoxyl 19.5, 19.7.

Some Chemical Properties of Trianhydrosucrose (X)

The compound (X) was heated at 100° in 25% aqueous potassium hydroxide for 24 hours. The solution remained

colorless and chromatography of a sample of the solution on paper showed compound (X) to have remained unchanged. When (X) was dissolved in 85% hydrazine and heated at 100° for 12 hours, the substance simply recrystallized unchanged. Compound (X) was dissolved in 0.01 N hydrochloric acid and the solution was kept at 23°. Aliquots were removed at various time intervals, neutralized with sodium hydroxide solution and the neutral solution applied to paper for chromatography (butanol-water). The substance was extensively degraded to materials which appeared as a streak, R_f 0.2-0.75, after 5 minutes of reaction time. Compound (X) was unaffected by Fehling's solution and was completely resistant to periodate oxidation at pH 8 and higher at room temperature for three hours. However, it did not survive treatment with periodate at pH 5. The substance, spotted on paper, was highly resistant to the periodate-permanganate spray reagent of Lemieux and Bauer (59). The ditosylate of (X), m.p. 158-159° (Calc. S, 10.75%, Found: S, 10.95%), was prepared in the usual manner. The compound was unchanged both by 10% sodium iodide in acetone and by 85% hydrazine at 100°. Methyl-2,4-di-O-mesyl-3,6-anhydro-α-D-galactoside has been shown (60) to be resistant to sodium iodide.

The Proof of Structure of Trianhydrosucrose (X) - The Isolation of 2,[redacted]-Di-O-methyl-D-galactose

The di-O-methyl derivative of (X), 50 mg, was dissolved in 1 ml of acetic anhydride and 0.03 ml of concentrated sulphuric acid at 0°. The solution was stored at 5° for 24 hours, during which time it developed a dark-green coloration. The solution was poured into an ice-water mixture to decompose the excess acetic anhydride then the aqueous mixture was extracted with chloroform. The chloroform solution was freed of acetic acid by washing with aqueous sodium bicarbonate (saturated) solution and dried over anhydrous sodium sulphate. Removal of the chloroform in vacuo left a syrupy residue, 45 mg, which was dissolved in 50 ml of hot water for deacetylation by percolating the solution through a column (45X8 mm) of strongly basic resin (Dowex 1X10) followed by elution with 400 ml of water. Evaporation of the water in vacuo gave a syrup which showed components with R_f values of 0.29, 0.36 (trace), and 0.61, when chromatographed on paper (butanol-water) using aniline phthalate spray reagent (53) to detect the position of the material on the chromatograms. The mixture, 28 mg, was added to the top of a Celite column (500X20 mm) for the partition chromatography using n-butanol-water system in the usual manner (54). The effluent was collected in successive 5 ml

fractions and the component with R_f = 0.61 was detected in fractions 19 to 23 and that of R_f = 0.29 in fractions 37 to 43. The appropriate fractions were combined and reduced to dryness in *vacuo*. In each case, the residue crystallized. The substance of R_f = 0.29, 10 mg, possessed an infrared spectrum identical in every detail to that of an authentic sample of 2,4-di-O-methyl-D-galactose (61). The identity of the material was confirmed by the preparation of its anilide, m.p. 214.5-216°, $[\alpha]_D$ +162° (c, 0.1 in pyridine), the melting point of which was unchanged by admixture of an authentic sample of the anilide (61) of 2,4-di-O-methyl-D-galactose, m.p. 215.5-216°, $[\alpha]_D$ = -183° (pyridine). The infrared spectra of the two anilides were identical. The component of R_f = 0.61, obtained in 15 mg yield, undoubtedly was 2,4-di-O-methyl-3,6-anhydro-D-galactose which survived the acetolysis, since the compound was converted to 2,4-di-O-methyl-D-galactose (identified by paper chromatography) by the procedure described above for the acetolysis of the di-O-methyl derivative of trianhydrosucrose (X).

The Tosylation of Sucrose using Sodium Hydroxide

The Tritosylation of Sucrose by the Usual Schotten-Bauman Procedure

Sucrose, 34.2 g, (0.1 mole), was dissolved in 60 ml

of 6 N sodium hydroxide (25% solution) and the solution was cooled to 0° using an ice-water bath. Tosyl chloride, 57 g, (0.3 mole) was dissolved in 50 ml of a suitable solvent (see table IV page 50), and added dropwise over a period of 30 minutes to the vigorously stirred aqueous phase of the reaction. The reaction vessel was kept half submerged in the ice-water bath and the stirring was continued until 30 minutes after completion of the addition of the tosyl chloride solution. The pH of the solution was tested with pH Hydrion paper from time to time and additional 10 ml aliquots of the 6 N sodium hydroxide solution were added when required to maintain the pH of the reaction well above 10. After the completion of the reaction time, the excess sodium hydroxide remaining was titrated with standardized 6 N sulphuric acid to estimate the amount of base consumed during the reaction.

The amount of base consumed in excess of 0.3 moles was a measure of the competing hydrolysis reaction and perhaps also the detosylation reaction (which both require in the overall reaction two moles of base per mole of tosyl chloride involved). Consequently, the number of moles of tosyl chloride that are involved in the esterification reaction only and that can account for the yield of ester may be estimated. The product was isolated by extraction with

chloroform, 300 ml. The chloroform was washed with water, 200 ml, dried over anhydrous sodium sulphate and evaporated in vacuo. The results of six such experiments are reported in Table IV, page 50, with the various conditions of reaction. Other experiments, where other solvents and operation conditions were used, gave even poorer results and are not reported here.

To estimate the amount of unreacted sucrose present in the aqueous phase (and washings) of reaction I, the following procedure was followed. The optical rotation of the aqueous phase was measured, + 7.25 (average of 5), and from the specific rotation of sucrose in water taken as + 66.7°, the concentration of sucrose per 100 ml of solution was calculated from the formula;

$$[\alpha]_D = \frac{\alpha \times 100}{l \times c} = +66.7 = \frac{7.25 \times 100}{2 \times c}$$

where c = g per 100 ml

l = length of tube (dem)

α = observed rotation

hence

$$c = \frac{+7.25 \times 100}{2 \times 66.7} = 5.41 \text{ g per 100 ml.}$$

Since the total volume of solution was 275 ml, the total amount of unreacted sucrose was 14.88 g. This

TABLE IV

The Tosylation of Sucrose in the Presence of Sodium Hydroxide

Reaction No.	1	2	3	4	5	6
Solvent for the tosyl chloride (60 ml)	Dioxane	Dioxane	$CHCl_3$	Dioxane	Dioxane	Dioxane
Time of addition (min.)	30	30	20	30	30	1
Reaction time after addition (min.)	30	15	180	30	30	10
Base consumed (moles)	0.430	0.342	0.350	0.416	0.43	0.400
Yield, (g) *	55	37	50	53.9	53.7	48
Sulphur content (%) calc. 11.93%	13.42	14.21	14.34	12.33	13.21	13.6

* The theoretical yield, based on the quantities of sucrose (0.1 mole) and of tosyl chloride (0.3 mole), is 80.4 g.

represented 43.5% of the sucrose present at the start of the reaction (34.2 g).

The Tosylation of Sucrose Using a Celite Column to Support THe Aqueous Phase

A - With Three Moles of Base per Mole of Sucrose

Sucrose, 34.2 g (0.1 mole) and sodium hydroxide, 12 g (0.3 mole), were dissolved in 40 ml of carbon dioxide - free water and completely mixed with 50 g of dry acid-washed Celite 535. This was packed in a 40 mm diameter column as a slurry in chloroform. Tosyl chloride, 60 g (0.3 mole), was dissolved in 90 ml of chloroform, the approximate volume required to displace all the chloroform already on the column. The tosyl chloride solution was forced through the column rapidly until the tosyl chloride solution was uniformly distributed throughout. The column was left aside for 18 hours and then eluted with chloroform, 600 ml. The product was found to be contaminated with a slight amount of unreacted tosyl chloride. Pyridine, 5 g (.06 mole), was added to destroy the tosyl chloride (slight evolution of heat). The cold chloroform solution was then washed with N sulphuric acid, 700 ml and then with 200 ml of a saturated solution of sodium bicarbonate. After drying

over anhydrous sodium sulphate, the chloroform solution was evaporated in vacuo. The yield was 43.8 g, 54.3% (theory 80.4 g). Silicic acid-impregnated paper chromatography of this product gave spots at R_f 0.8 to 0.95 indicating a high degree of tosylation.

B- With One Mole of Base per Mole of Sudrose

Sodium hydroxide, 4 g (0.1 mole), and sucrose, 34.2 g (0.1 mole) were dissolved in 20 ml of water. This solution was thoroughly mixed with 50 g of acid-washed Celite 535 and slurried into a 4 cm diameter column using chloroform. Tosyl chloride, 19 g (0.1 mole) was dissolved in 50 ml of chloroform and added to the top of the column. Elution of the column was controlled at 1 drop per second. Heat was evolved at the front of the advancing tosyl chloride solution so that its progress down the column was easily followed. One hundred ml of chloroform was collected before the first traces of unreacted tosyl chloride appeared in the eluent (2 hours). The next 100 ml of eluent was found to contain only tosyl chloride, 4.3 g (25%). The next 200 ml of eluent contained 6.7 g of highly tosylated tosyl ester, Fraction A. The following 200 ml of chloroform contained no product. Elution with 400 ml of acetone produced 12.5 g of tosylsucroses Fraction B

somewhat contaminated with inorganic salts. Silicic acid impregnated paper chromatography of Fraction A gave a streak from R_f 0.65 to 0.87 denoting a high degree of substitution while fraction B gave only two spots at R_f 0.04 and 0.15, probably corresponding to mono- and di-O-tosylsucroses.

DISCUSSION OF RESULTS

1. - A Chromatographic Analysis of the Tosyl Esters of Sucrose

"Tritosylsucrose", samples, prepared by the method of Hockett and Zief (2) both at 0° and at -18° were amorphous products. Their chemical heterogeneity was established by chromatography on paper impregnated with silicic acid (51) using diisobutylketone:acetic acid: water as solvent (35). The zones were located by dipping the paper in an aqueous solution of Rhodamine 6G (51). Fluorescent spots were observed under ultraviolet light at R_f values of 0.15, 0.40, 0.65, and 0.90. It was established that these spots corresponded to zones of di-, tri-, tetra- and penta-O-tosyl-sucroses, respectively, by paper chromatographic examination under the same conditions of the components which were isolated by preparative chromatography on a column of silicic acid using the same solvent system and which were analyzed. This paper chromatographic technique proved extremely useful for a rapid preliminary inspection of a product and the establishment of an appropriate solvent system for column chromatography.

The column chromatograms allowed the isolation of fractions that require the so-called "tritosylsucrose" prepared at 0° to be, chemically, a highly heterogeneous substance containing penta-, tetra-, tri-, and di-O-tosyl-

sucroses in the molar ratios 0.05: 0.33: 1 : 1, respectively (see Table I page 30). The tri-O-tosylsucrose fraction could be resolved into two sub-fractions which comprised 29% (tri-O-tosylsucrose A) and 13% (tri-O-tosylsucrose B) by weight of the original "tritosylsucrose". Both these fractions have thus far resisted crystallization. Their sulphur contents were in good agreement with that expected for sucrose tritosylates. That the substances were in fact different was obvious from the following properties. Whereas the A-fraction gave a yellow fluorescence with Rhodamine 6G, and readily consumed 2.82 moles of periodate per mole, the B-fraction produced a pink fluorescence and consumed only 1.75 moles of periodate per mole. Furthermore the A-fraction and the B-fraction underwent replacements of 1.87 and 1.63 tosyloxy groups, respectively, when treated with sodium iodide in acetone. These results clearly indicate that both fractions are mixtures of tritosylated sucroses. However, the high periodate comsumption by the A-fraction shows that it must be mainly (about 78%) 1',6,6'-tri-O-tosylsucrose. This conclusion was substantiated by the formation of 1',2;3,6;3',6'-trianhydrosucrose in 77.4% yield on treatment of the A-fraction with N sodium methoxide in methanol. These results provide unequivocal evidence that the trianhydrosucrose II (see figure 8, page 64)

obtained from the detosylation of the crude "tritosyl-sucrose", in fact arises from the 1',6,6'-tri-O-tosylsucrose.

The di-O-tosylsucrose fraction deposited a 10% overall yield (by weight) of crystalline compound (fraction C), m.p. 108-110°, $[\alpha]_D = +54°$ in ethanol. The sulphur content corresponded to that expected for di-O-tosylsucrose and the compound only reduced Fehling's solution after acid hydrolysis. The compound was acetylated and the acetate derivative, on treating with sodium iodide in acetone at 100°, produced a substance with an iodine content expected for diododideoxysucrose hexaacetate. Also, the nuclear magnetic resonance spectrum of the diiodo compound was devoid of the signals characteristic for the tosyl group. These properties require that the crystalline fraction C be the 6,6'-di-O-tosylsucrose. The syrup (fraction D) which remained after the removal of the crystalline 6,6'-di-O-tosylsucrose, had a sulphur content close to that expected for a sucrose ditosylate (see Table I). Treatment of the hexaacetate derivative of fraction D with sodium iodide in acetone replaced only 1.10 of the tosyloxy groups. This result shows that the 1'-hydroxyl group and perhaps certain secondary groups can compete favorably with the 6 or 6'-hydroxyl groups during the tosylation reaction. It will be seen later on that the

competition is undoubtedly mainly from the 1'-position and that, consequently, the di-O-tosylsucrose fractions (C and D combined) may be almost entirely composed of not too greatly different amounts of 1',6-, 1',6'- and 6,6'-di-O-tosylsucroses.

The substances isolated from the other two bands of the chromatogram possessed sulphur contents in good agreement with those expected for tetra- and penta-O-tosylsucroses. The results of the chromatogram are listed in Table I page 30 and the course of the tosylation is described in Figure 6 page 58.

A consideration of Figure 6 shows that the so-called "tri-O-tosylsucrose" does in fact contain 1',6,6'-tri-O-tosylsucrose as the main component. However, the compound is only present to an extent of about 0.29 moles per mole of sucrose reacted. The fact that this compound was formed in an amount more than twice that of any other tri-O-tosylsucrose shows that the 1'-position is substantially more reactive than any secondary hydroxyl group. Consequently, as was discussed above, the uncharacterised di-O-tosylsucrose (fraction D) can be expected to consist mainly of 1',6- and 1',6'-di-O-tosylsucrose. In this respect, it is of interest to note that Hockett and Downing(20) found 2,3;4,6-di-O-isopropylidine-L-sorbose to undergo tosylation half as

FIGURE 6

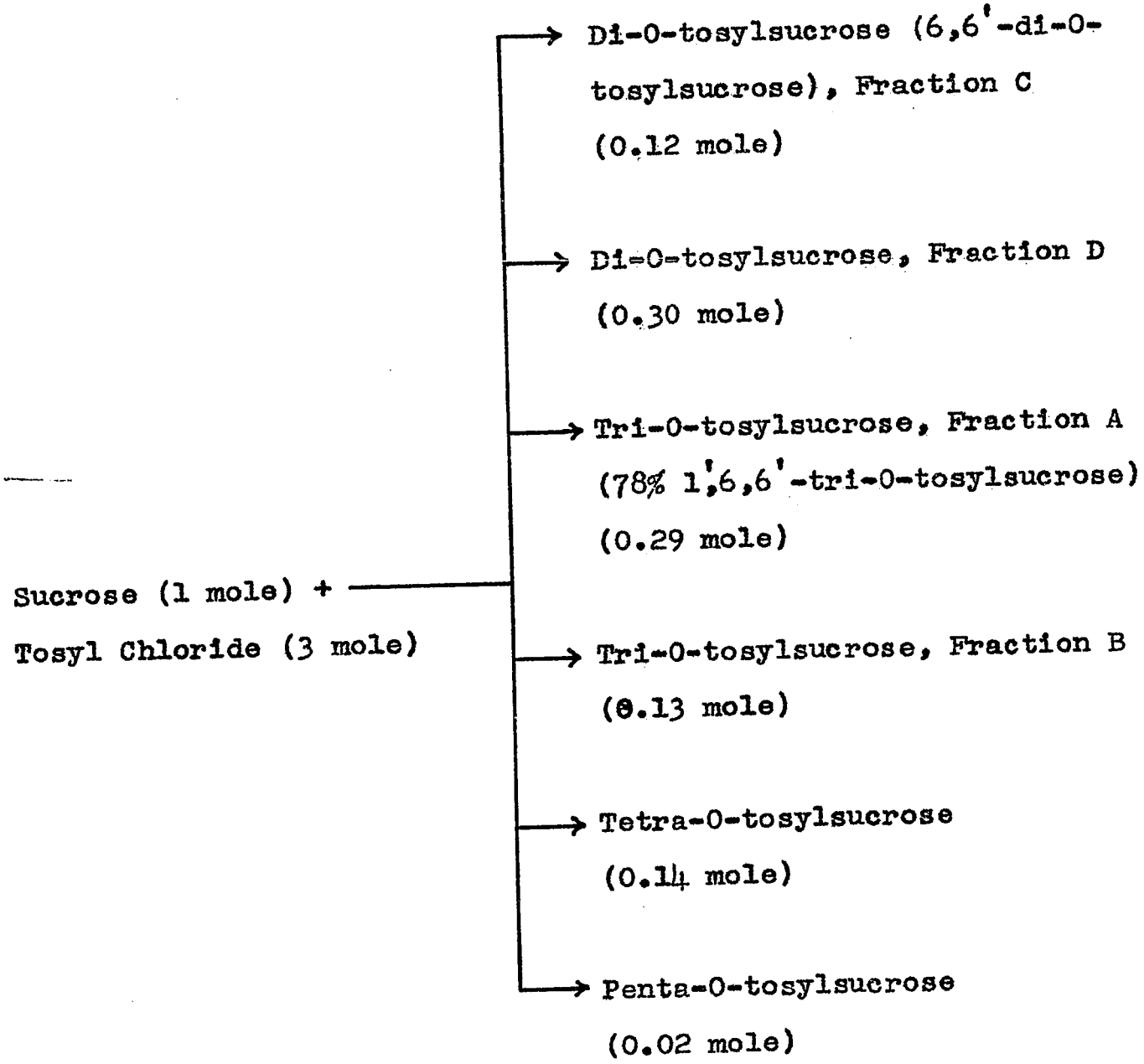


Figure 6 - Distribution of the tosylated sucroses formed in the reaction of one mole of sucrose with three moles of tosyl chloride in pyridine at 0°.

rapidly as 1,2:3,4-di-O-isopropylidene D-galactose (the former containing a primary hydroxyl group similar to the 1'-position in sucrose, the latter, one similar to the 6-position in sucrose). The results clearly render it unlikely that sucrose can be acylated preferentially in high yields at the 6 and 6'-positions only. The tritylation of sucrose has been shown (4) to yield 1',6,6'-tri-O-tritylsucrose pentaacetate in 45% yield. Table II page 31 describes the composition of the product obtained on a tritosylation of sucrose at -18°. It is seen that the course of the reaction was not appreciably affected by the lowering of the reaction temperature.

The product formed on the ditosylation of sucrose was analysed chromatographically. The results are presented in Table III page 32 and Figure 7, page 60. A comparison of the yields reported in figures 6 and 7 shows that the ditosylation produced, as would be expected, a greater yield of 6,6'-di-O-tosylsucrose (fraction C). The yield of the other sucrose ditosylates (fraction D) was, however, about the same as that obtained in the tritosylation. This result clearly indicates that the 6- and 6'-positions both undergo tosylation somewhat more rapidly than does the 1'-position. The fact that the ditosylation produced nearly as much 1',6,6'-tri-O-tosylsucrose

FIGURE 7

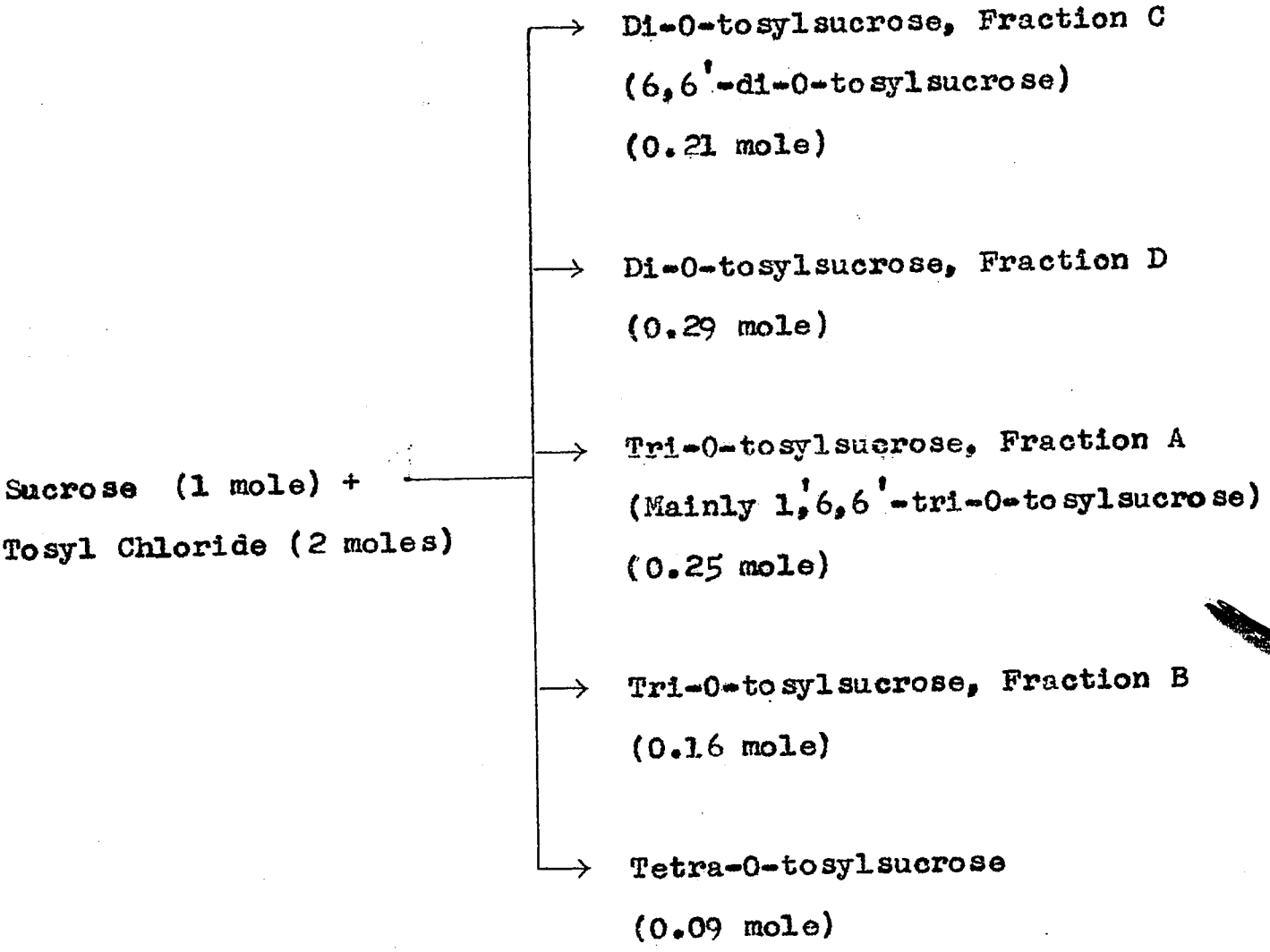


Figure 7 - Distribution of the tosylated sucroses formed in the reaction of one mole of sucrose with two moles of tosyl chloride in pyridine at 0°.

(fraction A) as did the tritosylation reaction clearly reflects the greater reactivity of the three primary positions over the secondary positions of sucrose. In conclusion, the above results show that although a substantial difference in reactivity between the primary and secondary hydroxyl groups in sucrose exists, the difference is not as great as earlier workers had anticipated and these results thereby correct a number of conclusions regarding the product of tritosylation of sucrose in pyridine, and the subsequent attempts to characterise the product.

2 - <u>The Detosylation of 1',6,6'-Tri-O-tosylsucrose. - The Formation and Proof of Structure of 1',2;3,6;3',6'-Trianhydrosucrose</u>

The formation of 1',2;3,6;3',6'-trianhydrosucrose (II) was anticipated on treatment of 1',6,6'-tri-O-tosylsucrose (I) with base (see figure 8 page). That this trianhydride was formed in high yield by the detosylation of the tri-O-tosyl-sucrose sub-fraction A is presented as evidence that sub-fraction A is in fact mainly 1',6,6'-tri-O-tosylsucrose. Also, in view of the chromatographic analysis of the crude "tritosylsucrose", it is not surprising that alkaline alcoholysis of the latter produced only low yields of the 1',2;3,6;3',6'-trianhydrosucrose (II).

That trianhydrosucrose II is in fact the 1,2;3,6;3',6'-trianhydrosucrose is obvious for the following reasons. The trianhydride II was extremely sensitive to acid hydrolysis, about two hundred times more rapid in 0.01 N hydrochloric acid at 25° than sucrose (52), measured polarimetrically. The initial rapid hydrolysis led to a reducing (Fehling's solution) compound (III), R_f value = 0.32 - 0.38 (butanol/water), which resisted crystallisation. The chromatographically pure product of hydrolysis III very rapidly consumed two moles of periodate without formation of either formic acid or formaldehyde. Since the trianhydride II resisted periodate oxidation at pH 7 or higher, it was evident that the acid hydrolysis had liberated two isolated α-glycol groups. Furthermore, the lack of formation of formic acid or formaldehyde showed the absence of -CHOH-CH_2OH, -CHOH-CHO, or -CO-CH_2OH groups. Since the acid hydrolysis of trianhydride II produced readily only one compound (III), it was apparent that the anhydrization had produced an ether linkage between the glucose and the fructose residues. It seemed likely that this linkage would be between the 2 and 1'-positions of the sucrose molecule since Helferich and Werner (62) have shown that the reaction of both the α and the β anomers of 2-chloroethyl D-glucopyranoside with alkali proceed with participation of the 2-oxygen atom to form 1,2-O-ethylene derivatives of D-gluco-pyranose.

The compound III was reduced with sodium borohydride to a non-reducing substance IV of R_f value = 0.20 - 0.24 (butanol/water) which, like compound III, very rapidly consumed two moles of periodate. Again no formic acid or formaldehyde was liberated during this oxidation.

The acetolysis (63) of IV and subsequent deacetylation gave a crystalline dihexitol ether V m.p. = 110-114° (dimorphic), $[\alpha]_D$ = -12.6° (in water) which on periodate oxidation produced two moles of formaldehyde, five moles of formic acid and an aldehydic residue VI which was reduced with sodium borohydride to 2-(β-hydroxyethoxy)-1,3-propanediol (substance VII). The identity of this triol (VII) was established by its synthesis through the periodate oxidation and subsequent sodium borohydride reduction of polygallitol (1,5-anhydro-D-glucitol (VIII). These results established the existence of the 1,2'-aliphatic type ether linkage between the anhydrohexopyranose and the anhydroketofuranose residues of the trianhydride II.

The infrared spectrum of trianhydride II gave no indication of unsaturation and the absence of epoxide ring was indicated by the substance's resistance to prolonged treatment both with 25% alkali and 85% hydrazine at 100°. The ditosylate derivative of trianhydride II, m.p. 164.5-166° dec., was unaffected by either sodium iodide in acetone or hydrazine at 100°.

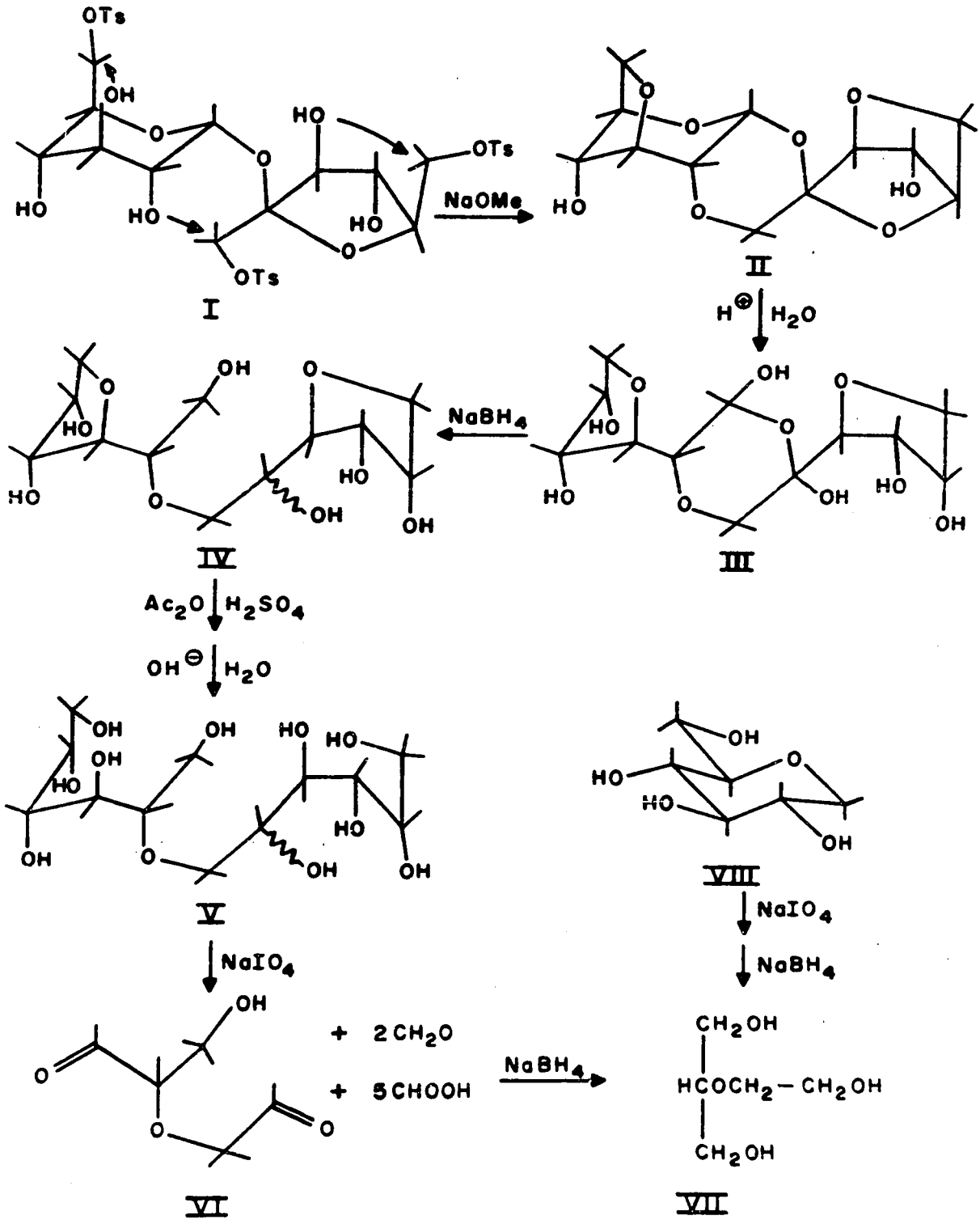


Figure 8. - The formation and proof of structure of 1',2;3,6;3',6'-trianhydrosucrose.

It could then be concluded that neither of the two free hydroxyl groups in II were at the 6- or 6'-positions.

The extremely rapid periodate uptake by compound III (see figure 8) and by its product of reduction (IV) (90%) uptake in 2 minutes in 0.02 N sodium metaperiodate at 0°) was clearly indicative of cis-α-glycols (58,64,65) situated on a five membered ring. Consequently, these results require that structures III and IV possess free cis-glycol groups at the 4,5 and 4',5' positions. Compound III showed no absorption in the infrared, characteristic of carbonyl groups. It seems probable that the carbonyl groups were masked through the formation of a hydrate as shown in formula for III (see figure 8).

The results require that both the glucose and fructose residues of compound III possess 3,6-anhydrorings. In view of these rings, the fact that the configuration at the 5 and 5'-positions were in all likelyhood not affected in the formation of trianhydrosucrose II, and the fact that 4- and 4'-positions (hydroxyl groups) are in cis-relation to the 5- and 5'-oxygens, respectively; it can be concluded that configurations at the positions 3,4,5,3',4' and 5' in trianhydride II are the same as in sucrose. Although methyl-3,6-anhydro- α-D-glucopyranoside is known readily

to undergo both α - to β -anomerization and pyranoside to furanoside ring contraction (45) under acid conditions; there can be no doubt that such anomerization would not occur under the alkaline conditions used to prepare the trianhydrosucrose II. Therefore, only the configuration at the 2-position of the glucose residue was reasonably in need of verification. Information on this point of structure was provided by nuclear magnetic resonance spectra of the diacetyl derivative of trianhydrosucrose II. Experience with nuclear magnetic resonance spectra of sugar derivatives (66) enables us to assign a doublet of total intensity one at 76.9 and 79.5 c.p.s. (60 Mc#s) (the lowest field signal in the spectrum) to the anomeric hydrogen of the glucose residue. The spacing for the doublet was found to be 2.6 ± 0.1 c.p.s. both at 40 and 60 Mc/s. This require that the spacing represents a true spin-spin coupling constant. The magnitude of the coupling is that characteristic for two neighboring hydrogens in gauche or skewed relationship (67). In view of the 3,6-anhydroring, the glucose residue must reside in that conformation which possesses the anomeric hydrogen in axial orientation. It follows, therefore, that the 2-hydrogen is in equatorial orientation since if it were in axial orientation, the coupling with the anomeric hydrogen would have been 2 to 3 times stronger (67) than that observed.

It follows on this basis that trianhydrosucrose II possesses the configuration of sucrose and it could be predicted that the compound was formed from 1,6,6'-tri-O-tosylsucrose. As was seen earlier on p. 55, the trianhydride (II) is in fact formed from 1,6,6'-tri-O-tosylsucrose. All attempts to obtain 4-O-methyl-D-glucose or 4-O-methyl-D-fructose by acetolysis (63) of the 4,4'-di-O-methyl-derivative of trianhydrosucrose II met with failure probably because of the high resistance of the 1,2 ether linkage to the acetolysis reaction.

It is of interest to note that titration of the solution obtained on periodate oxidation of hydrolysate III suggested that one mole of acid was liberated in the periodate oxidation. However, no formic acid could be detected in the mixture. The details of the efforts to characterize the formic acid are reported in the experimental section (p. 36) since they provide a convenient method for the micro estimation of formic acid and formaldehyde produced in periodate oxidations. Our results render obvious the need for the application of such a technique in experiments designed to elucidate points of structure in carbohydrate derivatives through a measure of the formic acid and formaldehyde produced in a periodate oxidation.

3 - The Detosylation of 1',4,6'-Tri-O-Tosylsucrose IX. - Formation and Proof of Structure of 3,6-Anhydro-α-D-Galactopyranosyl-1,4:3,6-Dianhydro-β-D-Fructoside. A Chemical Proof of the Configuration at the Anomeric Center of the Fructose Moiety of Sucrose

In an attempt to prepare larger quantities of 1',6,6'-tri-O-tosylsucrose, 1',6,6'-tri-O-tritylsucrose penta-acetate was prepared and detritylated to give the crystalline penta-O-acetylsucrose described by Hayward and coworkers (4). Although Hayward and coworkers had reported (4) that methylation of this sucrose pentaacetate had formed the 1',4,6'-tri-O-methylsucrose, they assumed that an acetyl group migration from position 4 to position 6 had occurred during the methylation reaction. However, we soon realized that the acetyl group migration probably had occurred during the removal of the trityl groups since tosylation of the resulting sucrose pentaacetate formed a tri-O-tosylsucrose pentaacetate IX which possessed only one tosyloxy group which was readily replaceable by iodine. Therefore the compound could possess only one tosyl group at either the 6 or 6' positions (see p. 43).

The tritosylsucrose pentaacetate could be assumed to have the tosyl groups at the 1',4, and 6'-positions of the sucrose residue in view of the well-established ease

for the 4 to 6 migration of an acetyl group on a glucose residue (68), no doubt by way of the orthoacid intermediate. This matter is dealt with in greater detail later on.

That the tritosyl derivative of Hayward's sucrose pentaacetate (4) possessed structure IX (see fig. 9 p. 73) became apparent from the structure of the trianhydrosucrose (X) which was formed in almost quantitative yield on its treatment with sodium methoxide in methanol. The structure of trianhydrosucrose X was established to be 3,6-anhydro-α-D-galactopyranosyl 1,4;3,6-dianhydrofructoside by means of the following experiments.

The trianhydrosucrose X reduced Fehling's solution only after acid hydrolysis. The substance was extremely sensitive to acid, undergoing rapid hydrolysis in 0.01 N hydrochloric acid at room temperature in a few hours. Trianhydrosucrose X consumed periodate at pH 5 but not at pH 8 or higher. The infrared spectrum of X gave no indication of unsaturation and this inference was supported by the compound's resistance to the periodate-permanganate reagent (59). The absence of 1,2-epoxide ring was indicated by the substance's resistance to prolonged treatment both with 25% alkali and with hydrazine at 100°. The ditosylate derivative of trianhydrosucrose X was unaffected by either sodium iodide in acetone or hydrazine at 100°. It could be concluded,

therefore, that neither of the two remaining free hydroxyl groups in X were at the 6- or the 6'-positions.

Methylation of trianhydrosucrose X gave a di-O-methyl derivative XI which was subjected to acetolysis (63) using concentrated sulphuric acid in acetic anhydride. Paper chromatography of the deacetylated product revealed the presence of three components (R_f values of 0.29, 0.36 (trace) and 0.61) which were separated by the method of partition chromatography on a column of Celite (54). The infrared spectrum of the compound in the main band, compound XII, (R_f value = 0.29), was identical to that of an authentic sample of 2,4-di-O-methyl-D-galactose (61). Since the substance with R_f value = 0.61 was converted to compound XII (R_f = 0.29) on reacetolysis and deacetylation of the product, it must be 2,4-di-O-methyl-3,6-anhydro-D-galactose.

The isolation of 2,4-di-O-methyl-D-galactose XII and its 3,6-anhydro-derivative established the trianhydride X to be a trianhydroepisucrose which possessed both remaining free hydroxyl groups in the 3,6-anhydro-D-galactopyranosyl residue. Since this residue must have been derived from the glucosyl portion of sucrose which is known (9) to possess the α-D-configuration, the 3,6-anhydro-D-galactopyranosyl residue of X must also possess the α-D-configuration.

Since the portion of "Trianhydrosucrose" X which was derived from the fructosyl portion of sucrose must

possess two anhydrorings, neither of which are of the 1,2-epoxide type, it follows that compound X must be the 3,6-anhydro-α-D-galactopyranosyl 1,4':3,6-dianhydro-β-D-fructoside.

Hayward and coworkers (4) found that methylation of the crystalline sucrose pentaacetate introduced O-methyl groups at the 1'-, 4- and 6'-positions. They suspected that an acetyl group migration from the 4- to the 6-position had occurred during the methylation reaction. It seemed probable however, that the sucrose pentaacetate was actually 2,3,3',4',6-penta-O-acetylsucrose. The fact that is is possible (69) to acylate 1,2,3,4-tetra-O-acetyl-β-D-glucose in pyridine (a compound which is known (68) to undergo acetyl group migration with great ease) without migration, suggests that the rearrangement would not occur during the tosylation of the sucrose pentaacetate. The tri-O-tosyl derivative (IX) of the sucrose pentaacetate underwent the replacement of only one tosyloxy group by iodine when heated to 100° with sodium iodide in acetone. In view of the results obtained by Hayward and coworkers (4, 70), the acetyl group migration from the 4-position to the 6-position must have occurred during the detritylation reaction (refluxing 30 minutes in 80% acetic acid) and the replaceable tosyloxy group must be the one at the 6'-position. The tosylated

derivative IX must therefore be the 1',4,6'-tri-O-tosylsucrose pentaacetate. This contention is confirmed by the fact that the formation of the trianhydride X can only be accounted for on the basis of this structure for IX, That is, the formation of the 3,6-anhydro-β-D-galactopyranosyl residue in trianhydride X would involve the formation of a 3,4-ppoxide ring (step 1, see figure 9) followed by migration of the epoxide ring (50) to the 2,3-position (step 2) to make available a route for the closure of the 3,6-anhydroring Step 3). Thus the "galacto-" configuration is achieved by inversion of C-4 (step 1) with two successive inversions at C-3 (step 2 and 3) leading to a net retention of configuration at this center. Helferich and Muller (71) have prepared a methyl anhydro-β-D-hexoside VIII m.p. 158°, $[\alpha]_D = -118°$ (water) by the alkaline methanolysis of methyl 4-O-tosyl-β-D-glucopyranoside triacetate. The structure of this substance has received considerable attention (72,73) and appears to be methyl 3,4-anhydro-β-D-galactoside (73). Our results clearly suggests that the methyl 3,6-anhydro-β-D-galactopyranoside should also have been a product of the reaction. Haworth, Jackson and Smith (74) have prepared this compound and report it to melt at 119° with $[\alpha]_D = -115°$ (water). The dimethyl ethers of these compounds are both crystalline compounds with m.p. 83-84°, $[\alpha]_D = -148.2°$ (chloroform) and m.p. 83°, $[\alpha]_D = -87°$ (chloroform),

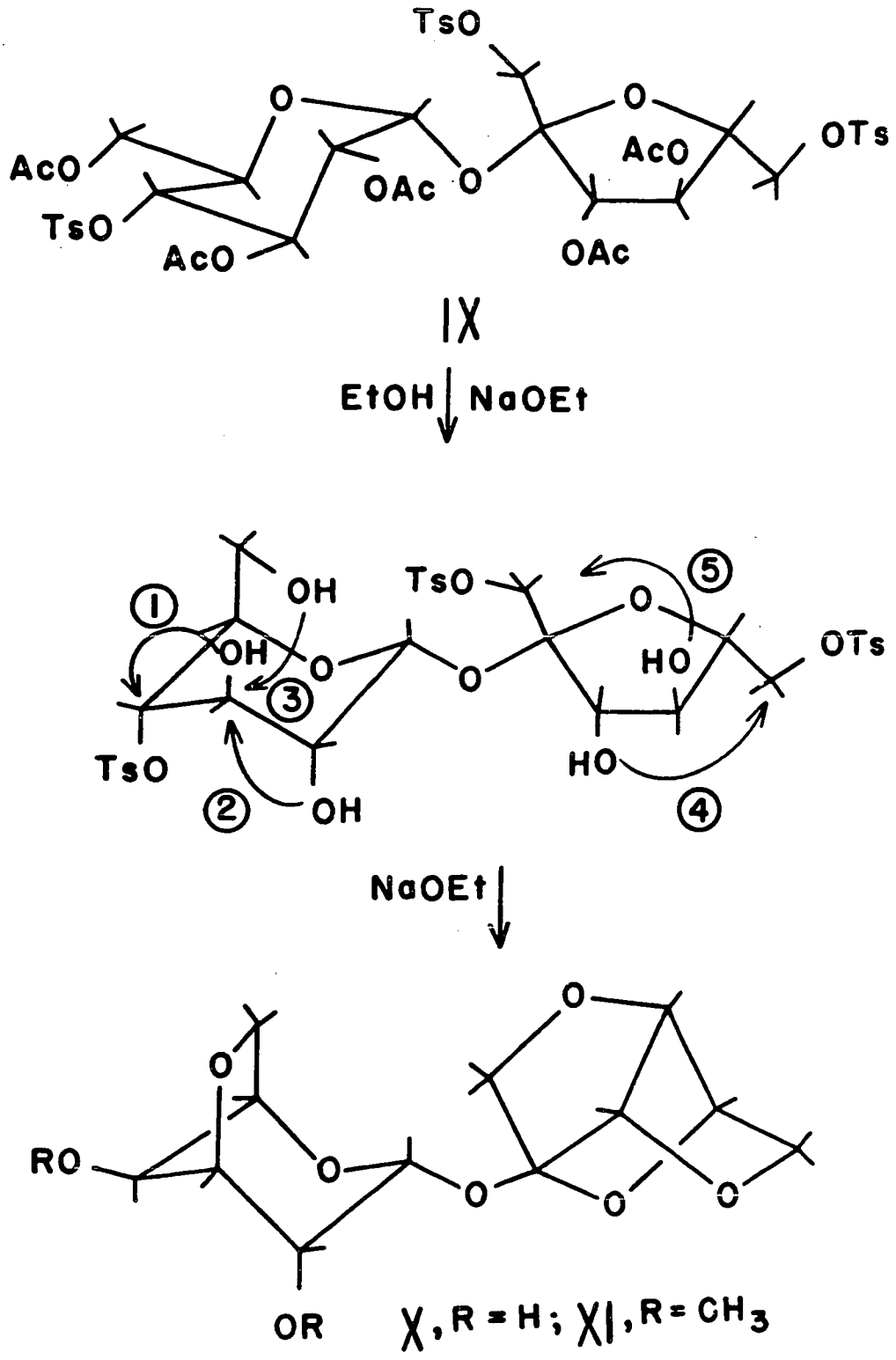


Figure 9. - The formation of 3,6-anhydro- ∝-D-galactopyranosyl 1,4;3,6-dianhydro- β-D-fructofuranoside - X.

respectively (74). Evidently, these compounds are not identical. The 3,4-anhydro-β-D-galactoside was prepared by extremely mild alkaline alcoholysis of the parent compound as compared with our conditions for the preparation of trianhydride X.

The steps 4 and 5 proposed for the formation of the 1,4:3,6-dianhydrofructofuranoside residue of trianhydride X are a well recognised type of reaction (75). However, the formation of this highly strained structure may not have been anticipated and provides an excellent illustration of the importance of anchimeric assistance in chemical reactions. It is of interest to note that the 1,4;2,5;3,6-trianhydro-D-mannitol prepared by Cope and Shen (76) possesses the structure of the 1,4;3,6-dianhydrofructofuranosyl residue of trianhydride X.

The formation of the 1,4;3,6-dianhydrofructosyl group of trianhydride X can only be accounted for on the basis of the β-configuration for the anomeric center of the fructosyl residue. Had the α-configuration been present, the formation of the dianhydrofructofuranosyl structure would have been impossible since the 1'- and the 4'-positions would have been oriented in opposite directions on the furanose ring and it would be impossible for the oxygen atom at the 4'-position to make a nucleophilic attack at the 1'-carbon atom. Therefore, the formation of trianhydride X

provides unequivocal chemical proof for the β-configuration of the anomeric center of the fructosyl residue of sucrose. The configurational assignment made on this basis is in agreement with that previously established by X-ray crystallographic studies (9). It does not seem well recognised that the previous chemical and biochemical evidence for the configuration of the fructosyl residue of sucrose (5) were ultimately based on the speculation that Hudson's rule of isorotation correlate configuration with rotation in the case of ketofuranosides as is now known to be the case for a variety of aldopyranoses and their derivatives (77). It is of real interest therefore that the present evidence for the structure of sucrose together with that previously obtained by X-ray analysis (9) allow the conclusion that the rules of isorotation in fact do correlate configuration with rotation in the case of fructofuranosides (78). In conclusion, it is noteworthy that the experiments discussed above complete the proof of the structure of sucrose by purely chemical means.

4 - <u>The Tosylation of Sucrose in the Presence of Aqueous Alkali</u>

Schotten (79) had introduced the use of sodium hydroxide in the role of acid acceptor in the benzoylation of amines, and Baumann (80) soon applied it in the benzoylation of alcohols and sugars. Later, Hinsberg (81) employed

aqueous alkali in the preparation of sulphonic esters. Essentially, the method consisted in shaking the sulphonyl chloride with a solution or a suspension of the alcoholic compound in sufficient concentrated aqueous alkali to neutralize the hydrochloric acid liberated in the course of the reaction. Menalda (82) made a quantitative study of this type of reaction and summarized the optimum conditions of the reaction.

Although this reaction has found only limited application in carbohydrate chemistry in view of its strongly basic condition, it was used successfully to tosylate partially substituted or "blocked" monosaccharides (83) and cellulose (84). It was therefore thought of interest to examine the composition of the products this type of reaction would form from the partial tosylation of sucrose using three moles of tosyl chloride per mole of sucrose in the reactions.

The results of the tritosylation reactions (using the usual Schotten-Baumann reaction conditions) are listed in Table IV, page 50. Chromatography of these products on silicic acid-impregnated paper revealed that the products were composed almost exclusively of highly esterified compounds, namely, penta-, hexa- and hepta-O-tosylsucroses. This is relatively easy to explain since it is very likely that a mono- or more extensively tosylated sucrose will undergo reaction at a much higher rate than sucrose itself.

This can be expected since the solubility of the first product in the non-aqueous solvent containing the tosyl chloride increases with each degree of substitution. From the amounts of base consumed in excess of that required for the esterification, it is seen also that considerable loss of tosyl chloride takes place through hydrolysis. The tendency for the pile-up of tosyl groups on relatively few sucrose molecules is reflected in the quantity of unreacted sucrose which remained at the end of the reaction. For example, in reaction 1, (see Table IV, page 50) approximately 43% of the sucrose still remained in the aqueous phase at the end of the reaction time. It seems, therefore, that only limited applications can be expected from the Schotten-Baumann type of reaction and in its simplest form. Therefore this type of reaction can be used effectively only when complete tosylation is desired. The use of homogeneous reaction conditions such as when pyridine is used as solvent are much preferable for partial tosylation.

CLAIMS TO ORIGINAL RESEARCH

1) - A proper characterization of the so-called "tritosyl-sucrose" was obtained by chromatography on silicic acid and has shown the substance to be a highly heterogeneous mixture containing not more than 30% 1',6,6'-tri-O-tosylsucrose. These results correct erroneous contentions made by earlier workers in this respect.

2) - Crystalline 6,6'-di-O-tosylsucrose was isolated and characterized for the first time.

3) - Crystalline 1',2;3,6;3',6'-trianhydrosucrose was isolated, characterized as the di-O-methyl, acetyl and tosyl compounds and shown to originate from the alkaline alcoholysis of 1',6,6'-tri-O-tosylsucrose. This is the first clearly established example of the cross-linking of the sugar residues in di- or polysaccharides.

4) - Crystalline 3,6-anhydro-α-D-galactopyranosyl 1,4;3,6-dianhydro-β-D-fructoside was isolated, characterized as the di-O-methyl, acetyl and tosyl compounds and shown to originate from 1',4,6'-tri-O-tosylsucrose. This provided an excellent example of epoxide ring migration and an unequivocal proof of the β-configuration of the anomeric center of the fructose moiety of sucrose and thereby completes the proof of the structure of sucrose by purely chemical means.

5) - The tosyloxy group situated on the 1'-position of sucrose was shown to be resistant to treatment with sodium iodide in acetone at 100°.

6)- A new compound, 2-(β-hydroxyethoxy)-1,3-propanediol, was prepared and characterized as the tri-O-acetyl derivative.

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