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PREPARATION AND STRUCTURES OF SUCROSE MONOESTERS

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PREFACE

The monoesters of sucrose prepared from the higher fatty acids have received much attention in recent years in view of their possible use as non-ionic detergents. The interest in these compounds was initiated and fostered by the Sugar Research Foundation, New York which has established grants in aid of research on these compounds in several consulting and university laboratories. The work presented in this thesis was part of this project and was concerned with the study of a variety of problems which arose in the attempt to prepare sucrose monoesters by esterification of sucrose using methyl esters with potassium carbonate as catalyst and N,N-dimethylformamide as solvent. The main concern was with the establishment of procedures for the isolation of pure reaction products, the analysis of crude reaction products, and the determination of the structures of the reaction products. Time did not allow a detailed study of the kinetics and thermodynamics of the reaction. However, a few preliminary results were obtained on these matters.

I wish to express my deepest gratitude to my research director, Dr. R.U. Lemieux, for trying to instil in me the proper approach so important in carrying out sound scientific research.

I also wish to thank the Sugar Research Foundation of New York for a grant in aid.

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ABSTRACT

Sucrose has been acylated by way of myristoyl chloride and also by transesterification with methyl myristate using potassium carbonate as catalyst in N,N-dimethyl formamide solution. The initial products in each case were highly substituted sucrose myristates. Under the conditions of the transesterification however, the polyesters reacted with the excess sucrose present to yield eventually sucrose monomyristates as the main product. The dimethyl formamide solvent did not react extensively with the sucrose to form formate esters.

Tosylation and iodination studies have shown that the 1'-tosyloxy group of a sucrose tosylate is not replaceable by an iodine atom, only tosyloxy groups at the 6- and 6'- positions react. This fact was used to show that approximately 50 percent of the ester groups of a sucrose monomyristate prepared by transesterification are at the 6- and 6'- positions of the sucrose residue. Periodate oxidations of these monoesters suggest that the other half of the groups occupy the 1'- position. Indications are that the sucrose monomyristates obtained from the acylation in pyridine have approximately 70 percent of the myristoyl residues at the 6- and 6'- positions of sucrose.

I. INTRODUCTION

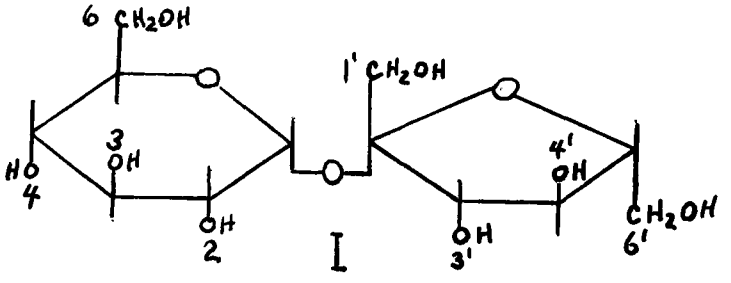
Since this research was concerned with the preparation of esters of sucrose for possible use as detergents, it was believed desirable to review briefly the variety of methods which have been used to prepare esters of carbohydrates together with the methods of isolation, purification and proof of structure of these compounds. Special emphasis was given to sucrose and its derivatives. The physical and chemical properties of sucrose have an important bearing on the possible application of these methods for the preparation of sucrose esters and the pertinent properties of sucrose are therefore also reviewed. A short note on the theory and classification of detergents is included.

1. Sucrose

Sucrose, β-D-fructofuranosyl α-D-glucopyranoside, occurs almost universally throughout the plant world. The principal sources of commercial interest are the sugar beet, the sugar cane and the sap of maple trees. Sucrose is a white non-reducing crystalline disaccharide melting between 160° and 188° C.(1) depending on the media used for its purification. The compound has a specific rotation, $[\alpha]_D^{20}$ +66.53° (c. 26 in water) (2), and is highly sen-

sitive to acids and enzymes undergoing hydrolysis in their presence to give a mixture of equal amounts of D-glucose and D-fructose. This hydrolysis process is called inversion by reason of the fact that the resulting mixture has a negative rotation.

Levi and Purves (3) have reviewed the literature relating to the structure of sucrose up to 1949, and the numerous reports of the biochemical synthesis of sucrose have been compiled by Hassid and Doudoroff (4) up to 1950. The configuration of the anomeric centers of sucrose were established by X-ray crystallographic analysis in 1947 (5). The conclusions reached in this fashion were substantiated by chemical means when Lemieux and Huber (6) synthesized sucrose in 1953. Recently, Lemieux and Barrette (7) have proven the β-D- configuration of the fructose moiety when they tosylated the crystalline 2,3,6,3',4'-penta-O-acetyl sucrose (8) and detosylated the resulting tritosylpentaacetate with alkali. The formation of 1,4;3,6 dianhydro-β-D-fructofuranosyl 3,6-anhydro-α-D-galactopyranoside was taken as chemical proof of structure I for sucrose.



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The numbering of the carbon atoms in the sucrose molecule(I) is that proposed by Hockett(9) in which the carbon atoms of non-reducing disaccharides containing glucose, are numbered by using plain numerals for the glucose moiety and prime numerals for the non-glucose part.

2. Detergents

a. Definition and Classification.- A surface active agent is one which modifies the properties of the surface layer of one phase in contact with another. In order to effect this change in energy between the two surfaces, a molecule must have two distinct portions, one of a hydrocarbon nature (hydrophobic) and the other of a polar solu bilizing nature (hydrophilic). At the interface between two immiscible substances the surfactant is oriented so that the hydrophilic portion is toward the polar phase and the hydrophobic portion is attracted by the non-polar phase. When multiple polar groups are present in a surface active molecule, they must be located at one end of the molecule. If they are a considerable distance apart, the desired orientation of the molecule at the interface is defeated. Compounds which are exceptions to this rule are usually limited as to surface active properties(10). It may be seen, consequently, that the properties of a surface active agent can be varied at will simply by changing

the lypophile-hydrophile balance.

The detergents can be divided into three classes; the anionic detergents are those which have the lypophilic portion as an anion, the cationic detergents are those which have the lypophilic portion as a cation, and the non-ionic detergents (11).

In the class of anionic detergents are found the ordinary sodium and potassium soaps, the purely organic soaps obtained when nitrogenous bases are used to form salts with fatty acids, and the alkyl aryl sulfonates. The organic soaps are excellent emulsifyers and good dry cleaning agents being soluble in organic solvents. Unlike the sodium soaps which cannot be used in acid media and in the presence of heavy metal ions, the sulfonates are sodium salts of strong acids and are unaffected by ions of magnesium, calcium, iron and other heavy metals, and also by low pH. An example is sodium dodecylbenzenesulfonate.

The so called "reversed soaps" which make up the second class, the cationic detergents, eliminate all interactions with heavy metal ions. They possess the disadvantage however of forming precipitates with ordinary soaps and with other long chain anions. These detergents

find use in acid and neutral media. They also exhibit marked bactericidal activity. The most common ones are quaternary nitrogenous compounds having at least one long chain alkyl group.

The non-ionic detergents overcome all the problems encountered by the reactions of ionic detergents with undesirable ions. There is however no case of a strongly hydrophilic non-ionic functional group; that property is supplied by more than one, usually several mildly hydrophilic groups. The main reasons for the growing market of non-ionics is that they are excellent wetting agents and produce low foam, a desirable property for automatic equipment. The non-ionics have also excellent cleaning properties. In this class of detergents enter the esters, ethers and thioethers of polyhydroxy alcohols and polyoxyethylene condensates. The polyoxyethylene esters produced from tall oil represent the largest fraction of these chemicals commercially made(12). The monoglycerides, the fatty esters of polyglycerol, pentaerythritol, sorbitol, mannitol, and of anhydro hexitols fall into this class.

It is only recently that fatty acid esters of sucrose have entered in the field of detergents. A considera-

tion of the availability and low cost of the raw materials entering in the manufacture of a sucrose fatty acid ester detergent, plus the fact that such a compound would hydrolyze in the stomach to form normal food components has renewed the interest in these compounds. Previously reported studies with polyoxyethylene condensates have demonstrated that for optimum surfactant properties, the presence of about two ethylene oxide units for every three carbon atoms in the alkyl chain is needed(13). One could expect that sucrose with eleven oxygen atoms would contribute about the same hydrophilic effect as a polyoxyethylene containing an equal number of oxygen atoms. An effective surfactant derived from sucrose would then require an alkyl group containing about 16 or 17 carbon atoms. This view was supported by the fact that glucose and sorbitol contain an insufficient number of oxygen atoms per molecule and that it was necessary to add oxyethylene groups to obtain sufficient water solubility with an alkyl chain of adequate size for surface activity(14).

b. Non-ionic Detergents Derived from Polyhydroxy Alcohols.- Even though the synthetic hydrophilic groups have a large part of the non-ionic field, the naturally occuring carbohydrates have been the object of continued research in the field of esterification.

The direct esterification of polyhydric alcohols with fatty acids is the oldest recorded and probably the simplest way by which to make polyhydric alcohol esters. It was back in 1860 that the French chemist Berthelot(15) tried to prepare an ester of sucrose by heating some sucrose with a fatty acid in a sealed tube. He obtained no reaction. However, even though some esterification occurs at ordinary temperatures, its progress is slow and long heating or high temperatures are required to carry the reaction to completion. It cannot be used therefore when there is danger of undesirable chemical changes such as polymerization, dehydration or charring. The greatest disadvantage of the method is observed when polyhydric alcohols are to be only partially esterified. In such cases, mixtures of more or less completely esterified alcohols are formed even if a large excess of the alcohol is used. The reason for this is that most polyhydric alcohols are not miscible with the fatty acid, even at elevated temperatures, while their ester products are. Thus the fatty acid reacts with the ester product in preferance to the alcohol yielding more highly esterified products than intended.

The case of the glycerides has been extensively studied. Belluci(16) reacted one mole of glycerol and one mole of fatty acid at 220°C. under a pressure of 30 to 40

mm. The bulk of the fatty acid disappeared in about two hours, but an excess of the glycerol remained. Monoesters form initially, but after the first half hour diesters prevailed. If the reaction was continued after all the fatty acid had reacted, the amount of monoglycerides again increased and the unreacted glycerol was used up.

Hilditch and Rigg (17) have shown that the esterification of ten moles of glycerol with one mole of fatty acid at 180° C. resulted in 70% conversion of the acid into ester after four hours. Only 46% of the esters isolated were monoglycerides. More recently, the direct esterification of methyl α-D-glucoside was reported (18). With a one to one molar ratio of the fatty acid to the glucoside, reaction times of ten hours at 230° C. gave diesters as main products.

The fatty esters obtained by treating hexitols with fatty acids at high temperatures are commercially important. The partial esters in particular have valuable surface active properties and their comparatively bland taste an freedom of toxicity give them a wide application in many fields (19).

In his attempts to esterify mannitol with lauric acid in concentrated sulfuric acid, Bloor (20) obtained a

mannitan dilaurate which he converted on heating to a dianhydro mannitol dilaurate. With 85% phosphoric acid as catalyst, Brown (21) obtained similar anhydro derivatives from the esterification of sorbitol and mannitol with fatty acids.

The above results show that the use of high temperatures and acid catalysts can cause deep changes in the alcohol molecules. Even if improvements have been made to better the yields and the quality of the products obtained by the direct esterification method, the conditions of this reaction do not allow sucrose to be esterified by this route since sucrose is highly sensitive to acids and temperatures above 100° C. will cause caramelization.

The use of acid chlorides and acid anhydrides in a large excess of anhydrous tertiary amine affords a favourable method for the esterification of carbohydrates under mild conditions. Usually the reaction is carried out in the cold or at room temperature.

In 1921, Hess and Messmer (22) obtained what they called octapalmityl sucrose by reacting sucrose with an eight molar excess of palmitoyl chloride in quinoline. The soft granular mass isolated was precipitated from ether by the addition of alcohol. The melting point of the ester

was 54.5° C. with $[\alpha]_D^{16}$ +17.12°. They also reported a sucrose octastearate. This solid, $[\alpha]_D^{16}$ +16.55°, was obtained as spherical microgranules, m.p. 57° C., from a chloroform-ethanol mixture.

Unsaturated fatty acid esters of sucrose have found application in drying oils, varnishes and artificial threads (23,24,25). Rosenthal and Lenhard (26) have prepared a polyoleate of sucrose by reacting sucrose with an excess of oleyl chloride. This ester, of which they do not give any analysis, was a limpid viscous oil soluble in benzene, terpentine, and linseed oil.

Harris (27) found that sucrose stearate, prepared from one mole of sucrose and one mole of stearyl chloride in pyridine, was an excellent anti-spattering agent for margarine. In 1934, Lorand (28) prepared sucrose palmitate using palmitic anhydride. Clayton and coworkers (29) have recently introduced the use of a variety of sucrose fatty esters as additives in lubricating oils. Although these authors list several fatty esters of sucrose such as a monopalmitoleate, dimelissate, tetraoleate and some mixed esters such as a sucrose monobutyrate dioleate and a monopalmitate dioleate, the preparative procedures were not reported. The fatty esters of octa 2-hydroxypropyl sucro-

se(30) have been studied as possible surface active agents.

The most widely used method for the preparation of polydric alcohol esters is that based upon the transesterification of fatty acid esters with the polyols. By eliminating the alcohol formed from the fatty acid ester, the reversible reaction can be made to go to completion.

The most thoroughly studied example of this reaction is the transesterification of alkyl esters of fatty acids with glycerol. Grün and coworkers(31) reported conversion yields of 94% to 96% when they reacted ethyl stearate with an excess of glycerol at 270°C. for periods of up to 15 hours. No catalyst was employed. Wright and coworkers(32) in 1944 found that the methyl esters of linseed oil fatty acids were transesterified readily with pentaerythritol when lead naphthenates were employed as catalysts. Temperatures of up to 280°C. were used at atmospheric pressure to obtain the conversion.

A systematic investigation was conducted by Gros and Feuge(33) in 1949 to compare the effectiveness of the various known catalysts for the alcoholysis reaction. Of the numerous catalyst studied, they found that barium hydroxide octahydrate, lithium hydroxide monohydrate, sodium ethoxide and sodium hydroxide were the most effective.

The reaction of 0.33 moles of glycerol and one mole of methyl fatty esters at 180°C. for two hours with six millimoles of sodium ethoxide gave the following product: 6.7% monoglycerides, 28.5% diglycerides, 35.5% triglycerides, 28.6% methyl ester and 0.68% free fatty acid. All the reactions seemed to come to equilibrium after 70% conversion. By employing an excess of methyl fatty esters, 90% conversions to triglycerides were obtained. The ethyl fatty esters were found to react less readily than the methyl esters.

Only a few attempts to replace the glycerol of triglycerides by higher boiling polyhydric alcohols have been reported. In the presence of sodium methoxide, olive oil and tristearin have been transesterified with mannitol by raising the temperature to 275°C. under a pressure of 14 mm. to remove the glycerol by distillation (34,35). Burrell (36) has studied the transesterification of a triglyceride (soybean oil) and pentaerythritol. The naphthenates of barium, cadmium, cerium, calcium, lead, lithium, strontium and zinc were effective catalysts.

Attempts to prepare the monooleate of methyl α-D-glucoside by heating the glucoside with olive oil at 225°C. with sodium methoxide catalyst finally gave an anhydro de-

rivative of the ester expected (37). More recently, the same problem was solved by transesterification with methyl oleate (38). A 72% yield of methyl α-D-glucoside monooleate was obtained after reacting 0.2 moles of the carbohydrate with 0.1 mole of methyl oleate at 230°C. for 30 minutes.

A modification of the transesterification reaction that has received little attention is the ester-ester interchange reaction. Konen, Cox, and Clocker (39) obtained pure trieleostearin by reacting three moles of neutral methyl eleostearate with one mole of anhydrous neutral triacetin in which 0.05 percent of dry sodium methoxide was dissolved. The reaction was conducted at 60° to 100°C. under vacuum.

Of the few neutral and acid catalysts (sulfonic acids and hydrogen chloride) studied, it is stated that they are too weak or ineffective. On the other hand, the alkaline catalysts have proven excellent for the transesterification reaction. In general, all alkaline substances capable of forming soaps as well as the soaps themselves are the strongest and most frequently used transesterification catalysts. The most widly used are the alkali alkoxides, alkali hydroxides and the alkali carbonates (40).

c. Detergents Derived from Sucrose. Although the preparation of fatty acid esters of sucrose by the acid chloride method has been the preferred procedure during the past 40 years, the transesterification of sucrose with methyl fatty ester has recently been receiving considera tion as a commercial approach to the preparation of these esters(41,42). The main reason for this choice is one of cost, while the acid chlorides are expensive, the methyl fatty esters are easily obtained from natural fats and oils. However, there are numerous difficulties. First, sucrose cannot be heated for prolonged periods at temperatures exceeding 100°C. without caramelizing. At safe operating temperatures the solubility of sucrose in fat is negligible. It is therefore necessary to employ a mutual solvent which does not decompose or enter into the reaction.

In 1956, F.D. Snell and coworkers(41) published a preparation of sucrose fatty esters by the transesterification reaction in which they employed N,N-dimethyl formamide (hereafter abreviated DMF) as a mutual solvent for the sucrose and the methyl fatty ester. Their first procedure consisted in heating, for three hours at 60°C., three moles of sucrose and one mole of methyl stearate dissolved in four liters of DMF. A 0.2 molar level of sodium methoxide catalyst was used. Under these conditions,

25 percent of the methyl stearate was converted to sucrose ester. Approximately the same yield was obtained when a glyceride ester replaced the methyl ester. It is clear that the above conditions did not take advantage of the fact that the methanol liberated during the transesterification is volatile and that removing it would cause the equilibrium reaction to go to completion.

The second procedure described by Snell(41) and the preferred one, involved the use of three moles of sucrose to one mole of a methyl fatty ester, with about 0.1 mole of an alkaline catalyst and sufficient DMF to dissolve the reactants completely. Potassium carbonate was found to be a suitable catalyst. Dimethyl sulfoxide was also used as solvent, but DMF was found superior. Potassium carbonate is more soluble in DMF than are calcium and magnesium hydroxides. Sodium carbonate is the least soluble of the four. All of these catalysts are insoluble in dimethyl sulfoxide(43). With sodium methoxide as catalyst, the use of temperatures sufficiently high for rapid stripping of the volatile alcohol resulted in undesirable side reactions.

The preparation of sucrose stearate is described as follows. Employing completely dry materials, the su-

crose was dissolved in the DMF (3.3 ml. of DMF per g. of sucrose) by heating with vigorous agitation. The methyl stearate and the potassium carbonate catalyst were then added and the reaction mixture kept at 90° to 95°C. at 80 to 100 mm. of pressure. A six plate fractionating column was suitable for stripping the methanol from the system. After nine to twelve hours, part of the DMF was distilled and the residue was dried under vacuum. Analysis of the reaction mixture after three hours of transesterification showed that the monoesters and diesters were present in approximately equimolar proportions. On further heating, the sucrose reacted with the diesters to form monoesters since after six hours, the molar ratio of monoesters to polyesters was about 2 to 1, and 23.5 to 1 after twelve hours. It appears therefore that the monoesters initially formed were esterified more rapidly than the sucrose present. However, the conversion of diesters to monoesters resulted in a product containing mainly monoesters. The amount of soap increased gradually. After twelve hours, almost 20 percent of the potassium carbonate present had been converted to soap. All the methyl stearate had reacted after four to six hours.

Using a lower level of DMF had no effect on the rapid initial esterification but slowed down the subsequent

conversion of diester to monoester. For example, the molar ratio of monoester to diester was reported as being 3.1 to 1 after seven hours but only 4.5 to 1 after a total of fourteen hours when 2.3 ml. of DMF were used per g. of sucrose in the reaction. The major effect of increasing the concentration of the catalyst was the formation of more soap as seen by the fact that when the concentration of potassium carbonate was increased sevenfold, approximately 50 percent of the methyl stearate originally present was converted to potassium stearate after twelve hours.

In a recent publication, Snell and Osipow(42) stated that after the methyl ester has been converted to sucrose esters by a procedure identical to the one described above, addition of some water to the reaction mixture will speed up the conversion of polyesters to monoesters. Thus, after a reaction lasting from three to six hours, maintaining the level of water in the mixture between 0.1 to 0.5 percent at 90°C. for an additional two hours resulted in the rapid conversion of diesters to monoesters.

The data given above by Snell and coworkers were made possible by an analytical procedure developed by these workers to study the mixtures obtained from the

preparation of sucrose esters(41). Their scheme of analysis was as follows.

Upon completion of a reaction run, a sample of the mixture was dried in a vacuum at 100°C. until constant in weight. Part of this sample was partitioned between n-butanol and 10% aqueous sodium chloride. Unreacted sugar is in the water layer. The optical rotations of both phases were determined. An aliquot of the butanol layer was titrated to determine soaps. Another aliquot of the butanol layer was taken to dryness to determine solids. Unreacted methyl ester was determined by saponifying the dry product, distilling the methanol and determining it colorimetrically with chromotropic acid(44). From the rotation of the water phase, the percentage sucrose present in the original mixture was calculated. From the observed rotation of the butanol layer was calculated the relative amounts of mono and diesters. This last operation requires the knowledge of the specific rotations of pure sucrose monoesters and diesters. The values of the latter were reported as being less accurate than those of the former. This analytical procedure was designed as a routine tool to assay reaction products rapidly. It does not comprise the isolation and analysis of pure products.

Several methods of purification for the sucrose monofatty esters were outlined by Snell and coworkers (41, 42). Of these, two are designed for the isolation of crude esters for commercial use while the third procedure leads to pure monoesters.

Procedure 1. After the reaction, the solvent was removed and the dry residue, which contained about 54 percent sugar, 1 to 2 percent potassium carbonate, and about 45 percent sucrose ester, was dissolved in three to four times its weight of water, and 5 percent sodium chloride (based on the water) was added. The mixture was then heated to 90°C. and maintained at this temperature until the sugar ester had completely layered. The curd was then withdrawn and dried. It contained 80 to 85 percent by weight sucrose ester with the remainder mainly sugar and salt.

Procedure 2.- The reaction mass, from which most of the DMF had been removed, was heated with ethy lene dichloride and filtered while hot to remove the sucrose, the catalyst, and small amounts of soap. The filtrate was then cooled to room temperature and filtered. The recovered filtrate contained any unreacted methyl ester and diesters. The washed filter cake was essentially pure

sucrose monoesters.

Procedure 3.- The complete removal of sugar and salt was accomplished by partitioning the solid, obtained after distillation of the DMF, between n-butanol and aqueous salt solution. Distillation of the butanol resulted in a product containing about 90 percent sucrose monofatty ester. The remainder was soap and sucrose polyesters. This product was recrystallized from acetone to give pure sucrose monoesters.

The evaluation of the surface active properties of the sucrose monoesters derived from lauric, myristic, palmitic, stearic and oleic acids has shown that these compounds are emulsifying agents and good detergents (45). Surface and interfacial tension measurements done between concentrations of 1.0 to 0.05 percent show that all the above esters lower the surface tension against Nujol, to between 8.4 and 5.0 dynes per cm. This is slightly better in lowering the surface tension than tall oil polyoxyethylene condensate and slightly less effective than sodium dodecylbenzenesulfonate. Wetting properties studied by the Draves (46) test indicated that the sucrose esters were only fair wetting agents. Sucrose laurate was somewhat superior to the other esters according to this method of evalua-

tion.

The sucrose esters were built for heavy duty detergency by adding 80 percent by weight of basic and neutral salt builders, and were evaluated separately for soil removal and for soil redeposition. The results indicated that the detergents from sucrose were equivalent to tall oil polyoxyethylene condensate and sodium dodecylbenzenesulfonate in soft water, but that the tall oil polyoxyethylene condensate was inferior to the sucrose esters and sodium dodecylbenzenesulfonate in hard water.

The foaming properties of the built sugar esters were measured by the method of Ross and Miles (47). The procedure involves measurements of foam heights produced under standard conditions. Results indicated that sucrose laurate and myristate are moderate to low foamers, while sucrose palmitate and stearate are low foaming agents. A study of the emulsions prepared with silicone oil and mineral oil and aqueous solutions of sucrose palmitate containing varying amounts of glycerol monostearate indicated that sucrose palmitate is sufficiently hydrophilic to require the presence of a lypophilic agent for good emulsification of non-polar oils.

The stability of the sucrose esters was determi-

ned by aging aqueous solutions of these compounds containing sodium tripolyphosphate. A 0.5 percent solution of sucrose stearate having a pH of 9.5 showed 8.9 and 14.5 percent hydrolysis after aging for one and four hours at 60°C., respectively. The stability in acid solution was studied by boiling a 0.1 percent solution of sucrose stearate in 0.1 N HCl. After thirty minutes, 6.9 percent of the ester had hydrolyzed. Feeding studies on rats showed that diets containing 10 percent sucrose monostearate caused no deleterious symptoms after one month.

3. The Structural Studies on Sucrose Esters

Unlike the fully substituted sucrose derivatives whose structures necessarily follow from that of sucrose, there are a large number of possible positional isomers for a sucrose derivative with any given degree of substitution. The problem of determining the percentage substitution of a group on each of the positions of sucrose for a monoderivative is a complex one. The only detailed structural study of a sucrose monoester reported in the literature is that of sucrose 2-phosphate prepared by treating sucrose with phosphorous oxychloride in lime water(48). The sucrose monophosphate was hydrolyzed in oxalic acid solution to give glucose phosphate, fructose and small quantities of fructose phosphate and free gluco-

se. Periodate oxidation showed that the phosphate group was attached to the second carbon of glucose. The structure of sucrose phosphate was then considered to be sucrose 2-phosphate mixed with a small quantity of another phosphate with the phosphate group in the fructose moiety.

The separation of the components of a mixture of a monosubstituted sucrose derivative into the positional isomers is a difficult task. A similar problem was partially solved by Asselineau(49), who studied the partial esterification of methyl α-D-glucoside and glucose with palmitoyl chloride in pyridine. Reacting one mole of the acid chloride with one mole of the sugars, the products isolated were separated on alumina and shown to contain only 55 and 45 percent of the 6 palmitoyl derivatives of glucose and of methyl α-D-glucoside, respectively. The remainder was separated into di, tri, and tetrapalmitates. The structure of some of these products was proven by periodate and lead tetraacetate oxidations, methylation studies, and also by the synthesis of some of the isomers using partially blocked glucose and methyl α-D-glucoside, for comparison purposes.

The separation of a mixture of the partially substituted derivatives of sucrose promised to be a more dif-

ficult task in view of the possibility of a greater number of components being present. It is therefore understandable that the only attempts to determine the structures of the positional isomers in a sucrose monoderivative were carried out on the mixtures. The properties of the sulfonate esters promised useful in this respect.

The p-toluenesulfonyl (tosyl) and the methanesulfonyl (mesyl) esters exhibit certain unique characteristics which make them of great importance in synthetic and analytical carbohydrate chemistry. Preparation of the sulfonates is accomplished by treatment of a carbohydrate with a pyridine solution of an aryl or alkylsulfonyl chloride ($R-SO_2-Cl$) or under Schotten Bauman conditions(50). Under these conditions all the hydroxyl groups may be esterified except those on the reducing carbons which are replaced by halide atoms. Thus glucose gives tetratosylglucosyl chloride. The primary hydroxyl groups are more easily esterified than the secondary hydroxyls(51,52,53). The Schotten Bauman reaction conditions for the preparation of the benzenesulfonates of sucrose were studied by Menalda(54) in 1930.

Only a few reports concerning the treatment of sucrose with p-toluenesulfonyl chloride are found in the

chemical literature. One of these was the treatment of 0.01 mole of sucrose with 0.03 moles of tosyl chloride in pyridine for twenty-four hours to yield an amorphous tri-O-tosyl sucrose (m.p. 66°-69°C.) having a specific rotation $[\alpha]_D^{27.6}$ +42.35° (c. 2.4 in $CHCl_3$)(55). This product was undoubtedly a complex mixture but Hockett and Zief postulated that due to the greater ease of tosylation of primary hydroxyl groups, the ester was mainly a 1',6,6'-tri-O-tosyl sucrose.

The tosyloxy groups which esterify primary hydroxyls may be replaced by an iodine atom when the ester is heated in an acetone solution of sodium iodine. The difference in ease of replacement of tosyloxy groups esterified with primary and secondary alcoholic groups is used to measure quantitatively the primary groups in a compound(56,57).

In 1944, Raymond and Schroeder(58) reacted 175 mM of sucrose dissolved in pyridine with 525 mM of tosyl chloride. The resulting tri-O-tosyl sucrose was treated with 58 g. of sodium iodide (386 mM) in acetone solution. The product of this reaction analyzed as a dideoxydiiodo mono-O-tosyl sucrose. These authors assumed that the three primary alcoholic groups of sucrose were esterified in the original sucrose tritosylate. A consideration of

the hindered nature of the 1'-tosyloxy group of the sucrose ester led these workers to believe that the 1'-tosyloxy group would probably resist replacement by an iodine atom. For this reason, they postulated that the iodo derivative was 6,6'-dideoxydiiodo-1'-O-tosyl sucrose. It is important to note that the conditions employed by these workers do not prove the resistance of the 1'-tosyloxy group to replacement, since only 2.2 moles of sodium iodide were used per mole of sucrose tritosylate during the reaction in acetone.

Tosyloxy groups at secondary carbon atoms usually remain unaffected by the sodium iodide in acetone treatment unless they are contiguous to a similar group esterified with a primary hydroxyl (59). When the latter condition exists, both groups may be removed with the formation of a double bond. Creation of a double bond also may occur when there is a free hydroxyl adjacent to a tosyloxy group at a primary position as in 6-tosylglucofuranosides (60). From the literature already described, it can be said with certainty that, since there are no secondary tosyloxy groups adjacent to primary ones in sucrose tosylates, the possibility of double bond formation during the treatment of these esters with sodium iodide in acetone is eliminated.

For their structural studies on sucrose monolaurate obtained by transesterification, Snell and coworkers (61) prepared a tosyl ester of the monolaurate containing 3.15 tosyl groups per molecule of laurate ester. Treatment of this tosyl derivative with a 2.64 moles of sodium iodide in acetone at 105° C. for 2.5 hours gave a sodium tosylate recovery indicating that 1.43 tosyloxy groups per mole had been replaced by iodine atoms. Similar treatment with four moles of sodium iodide for 16 hours resulted in 1.63 tosyl groups being replaced. Assuming three replaceable tosyloxy groups to be present on sucrose tosylate, these workers concluded that since low recoveries are not uncommon, the above iodination results suggested that the lauryl residues occupied only primary hydroxyl groups in the sucrose monolaurate studied. Thus, they disregarded the fact pointed out by Raymond and Schroeder (58) that the 1'-tosyloxy group of a sucrose tosylate is hindered and probably unchanged by the sodium iodide treatment.

The inversion of sucrose monolaurate in 0.5 N oxalic acid led Snell and coworkers (61) to conclude that 75 percent of the laurate group was on the glucose moiety of the sucrose monolaurate. This statement was based solely on the visual examination of paper chromatograms

done on the inverted products. The periodate oxidation of the same sucrose monolaurate resulted in an uptake of 2.915 moles of oxidant with the liberation of 0.682 moles of formic acid. Since substitution at the primary positions only can give a sucrose derivative consuming three moles of periodate, the oxidation of the monolaurate provided evidence to the effect that the lauryl groups were indeed attached almost exclusively at primary positions. Some uncertainty was introduced however by the low recovery of formic acid (theoretical value is one mole).

In summary, Snell and coworkers(61) concluded that the sucrose monolaurate prepared by transesterification was substituted almost exclusively at the primary positions, and that 75 percent of the laurate group was on the 6 position of the glucose moiety.

II. EXPERIMENTAL

1. Reagents

The N,N-dimethyl formamide (DMF) employed throughout this work was purchased from Eastman Organic Chemicals (b.p. 152° 154°C.). Azeotropic removal of any water present was effected by adding between one-quarter to one volume of dry benzene per volume of DMF followed by distillation at atmospheric pressure. The fraction collected between 152° and 154°C. had a refractive index of 1.4292. The recorded values for DMF are: boiling point: 153°C. and refractive index: 1.42938. This solvent could be distilled from phosphorous pentoxide but no advantage was gained by doing so.

The methyl myristate employed throughout this work was prepared from myristic acid (Eastman Chemicals m.p. 52°-53°C.) by esterification with an excess of dry methanol using concentrated sulfuric acid as catalyst. After refluxing for one hour, the ester layer was separated from the excess methanol by adding water and diethyl ether. The ether phase was dried with anhydrous sodium sulfate before distillation. The distilled methyl myristate had the following properties; boiling point: 147°C. at 6 mm., refractive index: 1.4371, saponification equiva-

lent: 240 g. and melting point: 18°-19°C. The recorded constants for methyl myristate are; boiling point: 155-7°C. at 7 mm., melting point: 18.5°C., and molecular weight: 242.4 g.

The myristoyl chloride was prepared from myristic acid and thionyl chloride in benzene solution according to the procedure of S.T. Bauer (62). The myristic acid (0.3 moles) was dissolved in 75 ml. of dry benzene by stirring at 50°C. in a 500 ml. three necked flask. The thionyl chloride (0.33 moles) was then added dropwise into the stirred solution. The mixture was heated at 55° to 60°C. for two hours. Upon removal of the solvent, much crystallization took place indicating that some acid was present. Another 0.33 moles of thionyl chloride were added and the mixture was refluxed for 3/4 hours. The benzene and the excess thionyl chloride were removed under a vacuum and the myristoyl chloride was distilled. An 85 percent yield of pure compound was collected between 155° and 156°C. at 8 mm. of pressure, which had a melting point of -1°C. The recorded constants for myristoyl chloride are; boiling point: 155°-157°C. at 7 mm. and melting point: -1°C.

The pyridine employed for the tosylations and acylations was the anhydrous reagent grade solvent having a

boiling point range of 1.4°C. It was distilled from phosphorous pentoxide. The p-toluenesulfonyl chloride, m.p. 66°-68°C., was the highest purity compound obtainable. The diethyl ether and the tetrahydrofuran employed for the lithium aluminum hydride reductions were dried over sodium metal and distilled from lithium aluminum hydride immediately before use.

2. The Acylation of Sucrose by Transesterification.

Using completely dry ground glass equipment and reagents, 179.5 g. of sucrose (525 mM) were dissolved in 600 ml. of dry DMF in a liter round bottom flask by heating and shaking the mixture. The methyl myristate (175 mM) and the anhydrous potassium carbonate (17.5 mM) were added to the sucrose solution. The reaction flask was then fitted with a Vigreux fractionating column. At the top of this column, an elbow was inserted which was in turn connected to a vacuum adapter fitted with a 200 ml. round bottom flask. A source of controlled vacuum was connected at this place.

For the transesterification, the reaction flask was immersed in a wax bath kept constant at 90°C.±1°C. A pressure of between 55 and 65 mm. of mercury was necessary to keep the reaction mixture refluxing strongly.

A Cartesian manostat was used to maintain the reaction pressure constant between ±0.5 mm. of the adjusted value. Under those conditions, the catalyst was usually completely dissolved after six hours. The temperature of the refluxing mixture was constant between 77°-79°C.

After completion of the reaction, the fractionating column was replaced by a Claisen head and the solvent was removed in vacuo. For this distillation, the reaction flask was kept immersed in a bath maintained at 90°C. The vacuum was gradually increased until the residue could be dried at full oil pump vacuum. The reaction cake was then dissolved in a one to one mixture of n-butanol and water, using approximately four ml. of this mixture of solvents per g. of reaction product. Sodium chloride was added to form a 5 percent aqueous solution and the n-butanol layer which separated rapidly was washed three to four times with half volumes of 5 percent aqueous salt solution. Each of these aqueous phases were in turn equilibrated with a second volume of n-butanol. The combined organic phases were then clarified with anhydrous sodium sulfate, filtered, and the crude ester product obtained by removing the solvent in a vacuum at 60°C. Identical results were obtained when methyl ethyl ketone was used in the place of n-butanol in the above isolation procedure.

3. The Acylation of Sucrose with Myristoyl Chloride.

To the 5 percent sucrose solution in pyridine kept at ice bath temperature, was added slowly with stirring the measured amount of myristoyl chloride. The precipitate that formed initially, dissolved more or less rapidly depending on the quantity of myristoyl chloride added. After standing 20 hours at room temperature, 3/4 of the pyridine was removed by distillation in a vacuum and the resulting solution was poured with stirring into a four molar excess of potassium carbonate (5 percent solution) based on the hydrochloric acid formed. An equal volume of n-butanol was then added and the esters of sucrose were purified of the excess sucrose by five extractions of the butanol phase with equal volumes of 5 percent aqueous sodium chloride. These aqueous phases were in turn extracted with a second volume of n-butanol. The combined organic phases were dried with anhydrous sodium sulfate, filtered, and the myristate esters obtained upon vacuum distillation of the butanol and the remaining pyridine.

4. Analytical Methods

a. Saponification and Fatty Acid Content Determination.- For the saponification of the sucrose esters, the micro method of Matther and Ziegenspeck(63) and the semi-

micro method of Mitchell, Smith, and Money(64) could not be used for solubility reasons. The saponification procedure employed throughout the present study was developed for the sucrose esters. It comprises a minimum of handling, requires only 0.5 m.e. of sucrose ester and is accurate to better than one percent.

The dried sucrose fatty ester (0.3 to 0.5 m.e.) samples were weighed into 150 ml. pressure bottles and exactly 25 ml. of 75 percent aqueous ethanol containing 1 m.e. of potassium hydroxide was added. The bottles were then heated in a water bath at 80° to 90°C. for approximately two hours. The excess alkali was titrated with 0.05 N hydrochloric acid to a phenolphthalein end point. The saponification equivalents were calculated in the usual manner using the difference between the titration of the blanks and that of the runs.

For the fatty acid content determination, the solutions from the saponification determinations were transferred into 250 ml. round bottom flasks, using ethanol to wash the contents quantitatively. The ethanol was then removed by distillation and the fatty acid precipitated by the addition of 100 ml. of cold water and 2 to 3 ml. of 2 N hydrochloric acid. After cooling, the fatty acid preci-

pitate was filtered and washed with cold water until free of chloride ions. The fatty acid on the funnel could be weighed (using a cintered glass funnel) and titrated in ethanol solution to the bromothymol blue end point (pH 6 to 7.6) with 0.05 N sodium hydroxide. Blanks, using the same volume of ethanol, were subtracted from the values obtained for these titrations. The standard base and acid used in the above procedures were prepared from carbon dioxide free water and standardized against potassium hydrogen phthalate.

b. Formyl Group Determination.- A procedure was developed during the course of this work to analyze the sucrose fatty esters for formyl groups. When known amounts of sodium formate were reduced with magnesium and hydrochloric acid, the colorimetric determination of the formaldehyde produced gave a measure of the sodium formate originally present. This was best accomplished by distilling part of the reaction mixture and doing the formaldehyde estimation on the distillate. This procedure, which is a modification of the method of W.M. Grant (65), was found to be easier to reproduce than the latter.

The method was standardized as follows. The sodium formate (between 0.05 and 0.7 mM) was dissolved in

in a 100 ml. round bottom flask using ten ml. of water. Concentrated hydrochloric acid (half an ml.) was added to displace the carbon dioxide and the flask was immersed in an ice bath. Magnesium metal, 0.6 g.,(30" 1/8" 1/5000") was added, followed by 4.5 ml. of concentrated hydrochloric acid added in 0.5 ml. aliquots at two minute intervals. After completion of the reaction, ten ml. of water was added and ten ml. of the solution was distilled into a ten ml. volumetric flask. For the colorimetric determination, one ml. aliquots of the distillate (or diluted solutions of the distillate) were treated with ten ml. of chromotropic acid reagent (66). The percent transmission was measured at 570 mμ in a Coleman Junior spectrophotometer using 19× 150 mm. cuvettes. Erythritol was oxidized with periodate and the formaldehyde produced (two moles per mole of glycol) was used to standardize the colorimetric method (66). The expression obtained was; number of millimoles of formaldehyde in a cuvette $= \frac{\log 1/T}{2155}$. The results of the control run with sodium formate are given in Fig. 1 as a plot of the millimoles of formaldehyde distilled against millimoles of sodium formate reduced.

The reduction of the sucrose myristates by the above procedure proceeded abnormally slow due to the poor solubility of the esters in the media. It became necessary

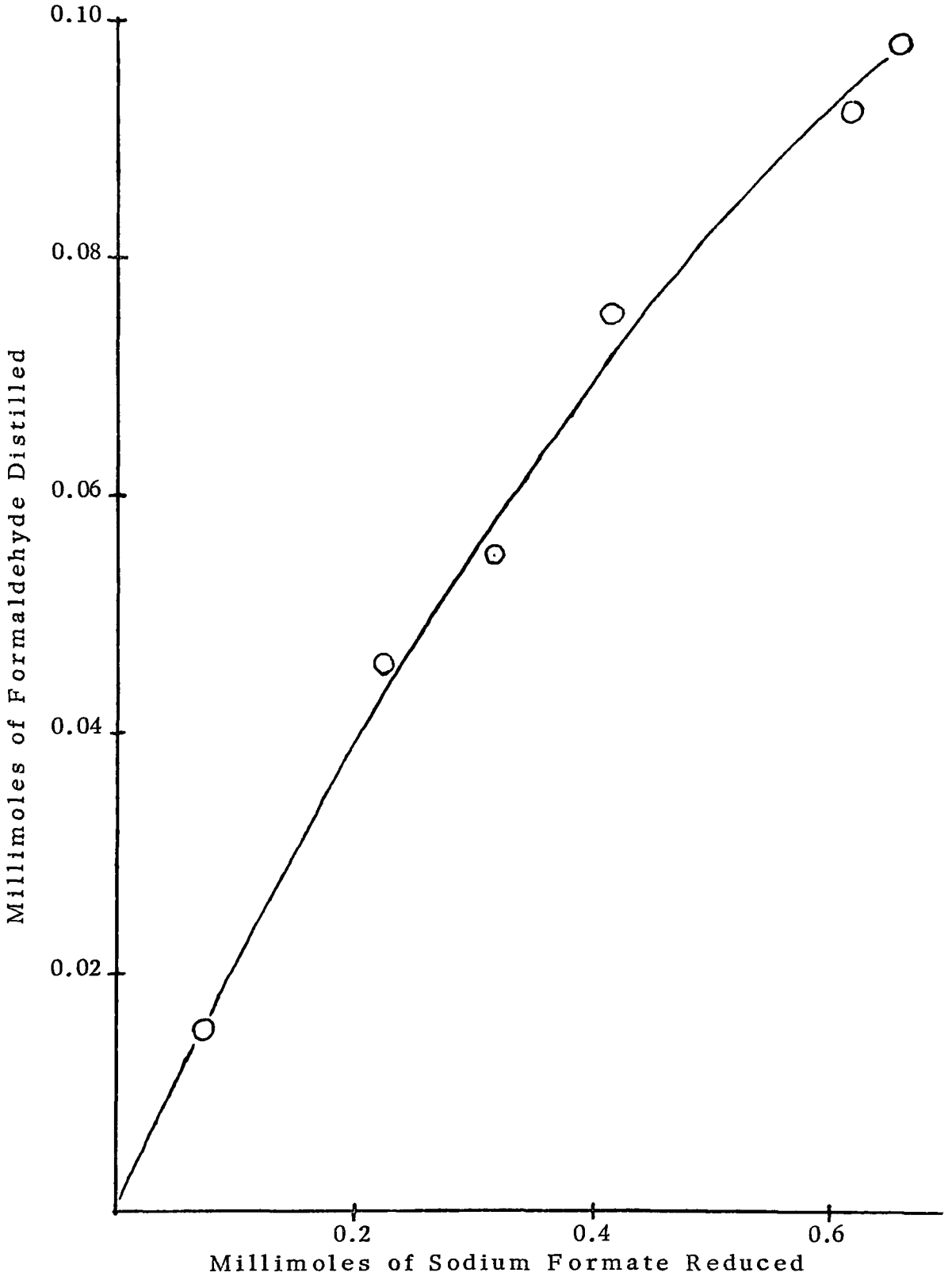

Fig. 1. Standard Curve for the Formyl Group Determination Procedure.

therefore to hydrolyze the esters of the fatty acid before carrying out the reductions. This was done by treating the aqueous solution of the ester (0.5 g. in 20 ml.) with four ml. of ten percent sodium hydroxide at 100°C. for 30 minutes. The fatty acid was then precipitated by adding five ml. of ten percent magnesium chloride solution. To facilitate the filtration of the magnesium myristate, 2 N hydrochloric acid was added to dissolve the magnesium hydroxide precipitate that had formed when the magnesium chloride was added. Phenolphthalein was added to make sure that the solution remained basic. The filtrate was collected in a 100 ml. round bottom flask, evaporated to dryness and the formyl content of the residue was determined as described for sodium formate.

Two control runs, simulating sucrose myristate samples, were made to test this procedure. Run A contained 0.3244 g. of sucrose, 0.1506 g. of myristic acid and 0.011047 g. of sodium formate. Run B contained 0.4565 g. of sucrose, 0.1814 g. of myristic acid and 0.059629 g. of sodium formate. The recoveries of formaldehyde were 66 and 61 percent of the amounts expected on the basis of the results plotted in Fig. 1.

c. Paper Chromatography of the Sucrose Esters.-

For this work, 8x22 inch sheets of Whatman paper No. 1 were used and the descending method was employed exclusively. The compounds chromatographed were added to the paper as one application of a four percent solution on points spaced 1.25 inches apart along the starting line, which was parallel to and 4.5 inches from the narrow edge of the paper. One application of a four percent solution approximately two cm. in diameter represents 200 micro grams of compound. After the applied spots had dried, the paper was placed in a chromatographic cabinet saturated with the bottom phase of the solvent system employed. The top phase was then added to the solvent trough and the front was allowed to move down 12 to 13 inches below the starting line. The paper was next dried for the spraying.

n-Butanol saturated with water, methyl ethyl ketone saturated with water and n-butanol: ethanol: water (5:1:4) were the most frequently used solvent systems. A number of spray reagents have been investigated for use with the fatty esters of sucrose on paper chromatograms. The hydroxamic acid spray reagent for detecting esters on paper chromatograms was tried without success (67). The following three reagents have found useful application during this work.

The aniline phosphate spray used was a modification of the reagent described by Bryson and Mitchell (68). The reagent was prepared by mixing before use, two volumes of 2 N aqueous phosphoric acid and one volume of 2 N aniline in ethanol, followed by three volumes of glacial acetic acid to dissolve the salt precipitate. The dried developed chromatograms were sprayed heavily with the solution, allowed to dry and then heated at 105° C. for 10 to 20 minutes. Any reducing pentoses and hexoses were detected as brown spots along with disaccharides (such as sucrose) which are hydrolyzed by the reagent to hexoses.

The glycol spray reagent of Lemieux and Bauer (69) was prepared by mixing before use, one volume of one percent potassium permanganate in two percent sodium carbonate and four volumes of two percent aqueous sodium periodate. The dried developed paper chromatograms were sprayed heavily with the solution and preferably placed in a humid atmosphere during the induction time. Glycols appeared as yellow spots on a purple background. Washing the excess permanganate-periodate off the paper resulted in a permanent brown spot on a white background.

The aniline hydrogen phthalate (70) spray reagent was prepared by dissolving 930 mg. of aniline and 1.6 g.

of phthalic acid into 100 ml. of n-butanol saturated with water. The dried, developed, paper chromatograms were sprayed with the reagent solution and heated at 105°C. for 10 to 20 minutes. This reagent is similar in selectivity to the aniline phosphate spray.

Although the sucrose fatty esters show much streaking on paper chromatograms, this technique has proven useful in obtaining qualitative information on a sucrose ester sample. Of the solvent system employed, n-butanol/water was found to give good resolutions and was easier to use than the others. With this solvent the R_f values of sucrose, glucose, fructose and sucrose monomyristate were found to be 0.045, 0.075, 0.10 and 0.75, respectively. Aniline phosphate was the preferred reagent for the fatty esters of sucrose. It had a higher sensitivity than the aniline phthalate reagent and gave chromatograms easier to read than those sprayed with the permanganate-periodate reagent.

A mixture of sucrose mono- and dimyristates was not separated on paper with n-butanol/water. Both compounds were found between R_f values of 0.7 to 0.85. However, during the spraying with aniline phosphate, the polymyristates of sucrose were not wetted by the reagent while

the monoesters were. Examination during the spraying therefore gave information regarding the composition of the mixture chromatographed.

d. Partition Chromatography on Celite.- The preparative partition chromatography of carbohydrates on Celite columns has recently been the subject of a publication (71). The method used for the sucrose esters was essentially the same. The Celite 535 employed as the absorbent was treated with concentrated hydrochloric acid before use (71). The columns were constructed by first, wetting the Celite with the water phase, using a one to one volume to weight ratio, slurrying this mixture in the developing phase and compressing into a chromatographic tube with a close fitting plunger. An approximately ten percent solution of the sample (preferably in the developing phase) was absorbed on dry Celite (1 ml. per g.) and the resulting powder was packed to the top of the column which was just filled with developing phase. The top surface was protected by a filter paper. The developing phase was then percolated through the column by gravity and the eluate was fractionated by an automatic collector. When two or more irrigating solvents were used in succession, the level of the previous solvent was just below the top of the column before the next solvent was added.

Since all the compounds chromatographed contained sucrose and sucrose derivatives, the fractions were tested for their sucrose content using the anthrone reagent (72). This reagent does not function properly in the presence of alcohols and ketones, and it was therefore necessary to free the samples of organic solvents before adding the reagent. This was conveniently done by placing the cuvette (19x150 mm.) of a Coleman Junior spectrophotometer, containing the aliquot to be analyzed, in a test tube immersed in a bath of oil heated to about 90°C. The bath was then placed in a large vacuum dessicator for evaporation in vacuo. Three ml. of water and six ml. of anthrone reagent were then added. It is to be noted that uniform and rapid addition of the anthrone reagent is necessary and this is best accomplished with a syringe pipette. The anthrone reagent was prepared by dissolving two g. of anthrone in a liter of concentrated sulfuric acid (72). The anthrone used was obtained from the reduction of anthraquinone with tin and hydrochloric acid (73).

After standing at room temperature for one hour, the green colour produced was read at 620 mμ. Sucrose was used to standardize the method. A typical run with sucrose is given in Table I. The sucrose content of a tube was calculated from this slope using the expression;

micrograms of sucrose $= \frac{\log 1/T}{7.555 \times 10^{-3}}$.

Table I

Standardization of the Anthrone Method for the Determination of Sucrose Contents.

Micrograms of sucrose in cuvette	Log 1/T	Slope
18.7	0.144	7.70×10^{-3}
37.4	0.282	7.54 "
39.8	0.304	7.65 "
69.3	0.518	7.48 "
74.8	0.564	7.54 "
149.7	1.111	7.42 "
	Average	7.555×10^{-3}

When the developing phase had not completely removed the components from the column, the latter was extruded and the positions of the bands were determined by spraying the column through a mask (74) with a one percent solution of potassium permanganate in 2.5 N sodium hydroxide (75).

Attempts were made to analyze the reaction mixtures of the preparation of sucrose myristate by transesterification using Celite partition chromatography. Pre-

liminary studies with the solvent systems n-butanol saturated with water and methyl ethyl ketone saturated with water showed that these solvents eluted the ester samples too rapidly. Other chromatograms with the solvent system heptane: methanol: water (15:9:1) showed that the heptane phase was eluting some polyesters of sucrose since the eluate contained a small carbohydrate band. However no separation of the mono- and diesters was achieved.

A chromatographic procedure was developed in which the columns were eluted with three consecutive solvents, namely, with heptane saturated with water, then with n-butanol, and, finally with water. The columns were prepared as a slurry with n-butanol saturated with water and then irrigated with heptane phase to remove the butanol. Two Celite columns (25 g. of Celite in 26 mm. tubes) were prepared in this way to analyze a mixture (25 mg.) of sucrose, sucrose monomyristates and of sucrose polymyristates. The columns were developed first with 125 ml. of heptane saturated with water, then with 100 ml. of n-butanol and finally with 100 ml. of water. The eluates were collected separately and analyzed for their sucrose content with anthrone. The recoveries were 93.5 percent. The results indicated the presence of 17-19% of the sucrose in the heptane fraction, 61-64% of the sucrose in

the butanol fraction and 20% of the sucrose in the water fraction.

The course of a transesterification reaction was followed using the Celite chromatographic method to analyze the reaction mixtures isolated after a variety of reaction times. Sucrose (75 mM) and methyl myristate (25 mM) were reacted with 2.5 millimoles of potassium carbonate in 86 ml. of DMF as described on page 31. The pressure was kept at 49 mm. and the temperature of the refluxing solution was constant between 76° and 77°C. The solution was sampled from time to time through a sampling tube without disrupting the reaction conditions. A 0.1 ml. volume of the reaction mixture was used for the chromatographic separation of the components on Celite columns, 25 g., 26 mm. in diameter. The carbohydrate contents of the three fractions were determined with the anthrone reagent. The exact amount of sucrose in the aliquots being unknown, the results given in Table II appear as the percentage sucrose in a given fraction to the total amount of sucrose found in all three fractions. The first fraction contained only traces (less than 1%) of sucrose derivative and is not reported in Fig. 2.

Table II

Chromatographic Analyses of the Reaction Products from the Transesterification of Methyl Myristate with Sucrose.

Time in hours	Percent Sucrose		
	Heptane fraction	n-Butanol fraction	Water fraction
0	0	0.6	99.4
0.5	0	11.2	88.2
1.0	0.17	17.3	82.7
3.3	0	31.2	68.8
5.3	0	39.9	60.1
8.3	0	34.0	66.0
10.6	0.35	39.1	60.55

5. Determination of the Number of Myristoyl Groups Occupying the 6 and 6'- Positions in a Sucrose Myristate.

a. The Preparation of the Tosyl Esters (76).- The carbohydrate sample to be tosylated was weighed into an oven dried ground stoppered flask and dissolved with dry pyridine. The level of the pyridine employed was based on the tosyl chloride used, 100 ml. of pyridine per 25 g. of tosyl chloride. This solution was cooled in an ice bath and a 10 to 25 percent excess of tosyl chloride was added. The solution was well stoppered and kept at

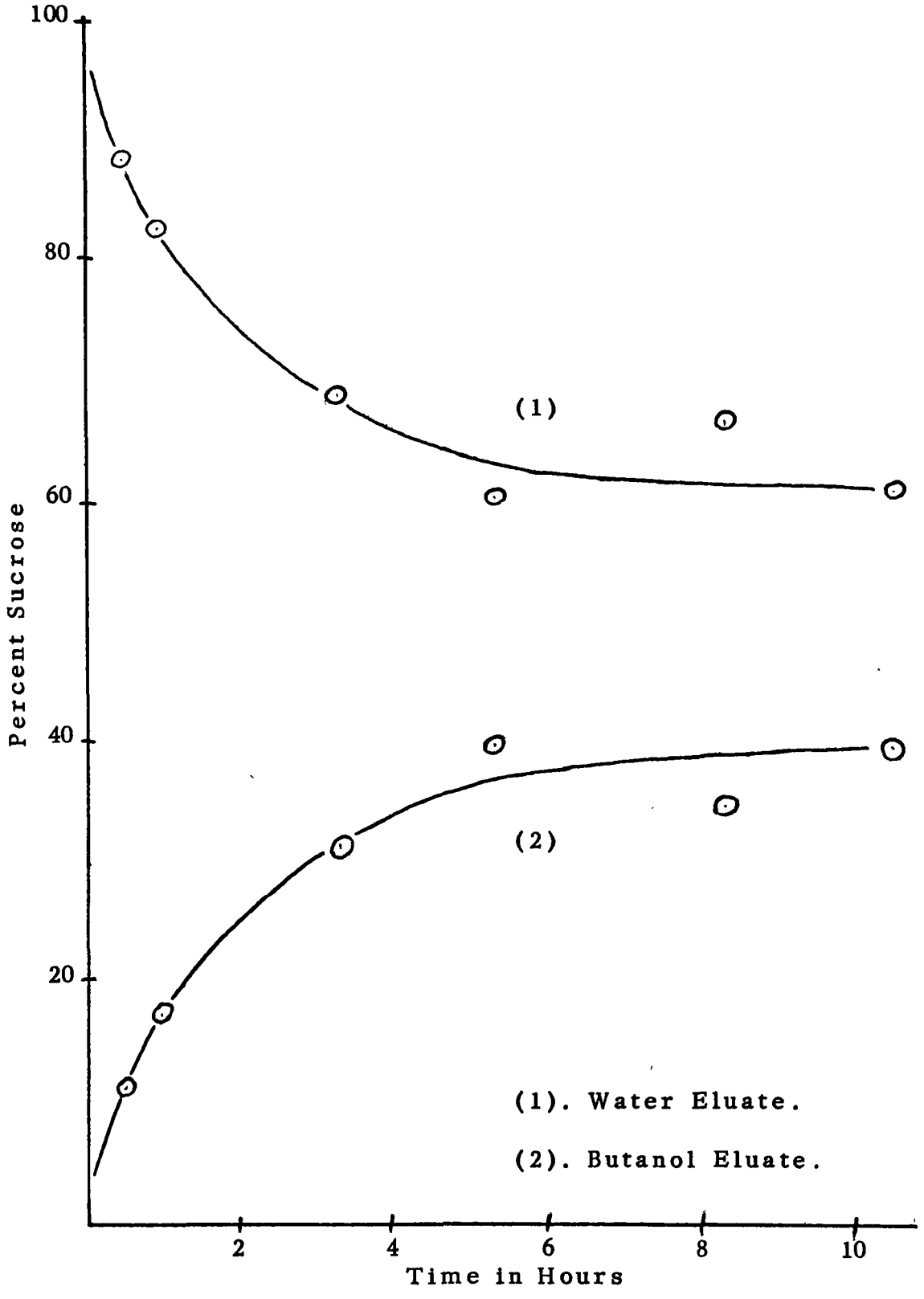

Fig. 2. Kinetic Run of the Transesterification Reaction.

5°C. for ten to eleven days. After this time, the pyridinium hydrochloride formed was removed by filtration and washed once with some pyridine. The excess tosyl chloride in the pyridine solution was destroyed by the slow addition, with shaking, of water equivalent to about half of the tosylating agent originally used. The solution was then evaporated to near dryness in a vacuum and the residue dissolved in chloroform (100 ml. of chloroform per 25 g. of product). The chloroform solution was extracted twice with 10 percent sulphuric acid, once with saturated sodium bicarbonate solution, washed with water, and dried with anhydrous sodium sulfate. The tosyl ester was obtained by evaporating the filtered chloroform solution to dryness.

The sulfur contents of the tosyl esters was determined by the method of Sundberg and Roger (77). The ester (10 to 40 mg.) was burned in an oxygen atmosphere and the gases were collected in a Grote absorber with three percent neutralized hydrogen peroxide. The solution was transferred to a 125 ml. Erlenmeyer, boiled to expel the carbon dioxide, and the sulfuric acid was titrated with 0.05 N sodium hydroxide to the methyl red end point. The sulfur analyses of 1,2-3,5-di-O-methylene 6-O-tosyl-D-glucofuranose (m.p. 112.5°-113°C.) by the above method gave values of 8.91, 8.82, 8.92, 8.96 and

8.97 percent sulfur. The calculated value is 8.95 percent sulfur.

The results of the tosylations and sulfur analyses are reported in Table VII.

b. The Iodination of Tosyl Esters (76). In all experiments carried out, the tosyl ester was weighed into a 125 ml. pressure bottle and dissolved with a ten percent solution of sodium iodide in acetone. This reagent (Finkelstein's reagent) was prepared by dissolving ten grams of sodium iodide in 100 ml. of acetone. Between 12 and 14 ml. of the sodium iodide solution were used per gram of ester, this amount being at least a twofold excess of sodium iodide over the tosyl compound. The sealed bottle was then heated in steam for the length of the reaction time. After cooling, the bottle was opened and the acetone evaporated. Equal volumes of benzene and water were added to the residue using 100 ml. of the solvent mixture for one to three grams of tosyl ester originally employed. A little sodium thiosulfate was added at this stage to reduce any free iodine present. The benzene layer was then washed once with water, dried with anhydrous sodium sul fate and the product was obtained by evaporation of the benzene solution to dryness.

The extent of replacement of the tosyloxy group was obtained from the iodine content of the iodo derivati ves. The sample to be analyzed (10 to 40 mgm.) was bur ned in an oxygen atmosphere and the liberated iodine collected in a Grote absorber with five percent sodium hydroxide (77). The contents of the absorber were transferred to a sodium acetate, acetic acid buffer and bromine was added to oxidize all the iodine absorbed to iodate. After destroying the excess bromine, the iodate was titrated in the usual way with thiosulfate. The iodine analyses of 1,2-3,5-di-O-methylene 6-deoxyiodo-D-glucofuranose (m.p. 96.5°C.) gave values of 41.85, 40.13, 39.91, 40.29, 40.18 and 40.80 percent iodine. The calculated value is 40.42 percent iodine.

The results of the iodination experiments are given in Table VII.

6. Sodium Periodate Oxidations (78)

The sugar (0.2 mM for hexoses and 0.1 mM for disaccharides) was weighed and dissolved in a 100 ml. volumetric flask with 75 ml. of water. At temperature equilibrium, 20 ml. of 0.1 M sodium meta-periodate were pipetted into the sugar solution and the volume was made up to the mark. To stop the reaction, 10 ml. aliquots of

the oxidation mixture was pipetted into a known excess of sodium arsenite (10 ml. of 0.05 N sodium arsenite) containing an excess of sodium bicarbonate and a few potassium iodide crystals. After ten minutes, the excess arsenite was titrated with 0.02 N iodine solution to a starch end point. The difference between this titer and that of a reagent blank was taken as a measure of the extent of oxidation. Table III lists the values obtained when glucose and sucrose were oxidized at 24.8°C. The pH of the blank run was 5.2 while that of the sucrose oxidation mixture after two days was 3.8. The uptake of oxidant shows that no hydrolysis of the glycosidic bonds occurred.

Table III

The Oxidation of Sucrose and Glucose with Sodium meta periodate.

Sucrose		Glucose	
Time in hours	mM periodate per mM.	Time in hours	mM periodate per mM.
0.3	1.65	0.3	3.38
0.8	2.28	0.8	4.63
3	2.70	3	4.96
4	2.85		
48	3.01		

The results of the oxidation of sucrose monomyristate are reported in Table IX.

7. Reduction of Tosyl Esters with Lithium Aluminum Hydride.

For the reductions carried out in diethyl ether, the tosyl ester was added, as a concentrated solution in dry benzene, to a five equivalent excess of lithium aluminum hydride slurried in ether. The reaction mixture, stirred through a mercury seal, was refluxed for 48 hours. The excess reagent was destroyed first by the addition of a little water followed by the addition of a large excess of water. The water phase from the filtrate of the above mixture was extracted with benzene to remove any p-ditolyldisulfide (79). The aqueous solution was then passed through a column of 200-400 mesh Dowex 1-X10 (quaternary ammonium type anion exchange resin) and then through a column of Amberlite IRC50(H) (a carboxylic type resin manufactured by Rohm and Hass Co.). The eluate issuing from the cation exchange resin always had a pH of 3.9 and boiling did not change this pH. Sufficient sodium bicarbonate was added to raise the pH to 7 before the evaporation of the water. The concentrate was then freed of the ions added by passing it through a small excess of the

two resins mentioned above. The product was obtained from this solution by evaporation of the water to dryness.

The reduction in tetrahydrofuran was carried out according to the procedure described for diethyl ether. The tosyl ester was dissolved in tetrahydrofuran instead of benzene for the addition to the lithium aluminum hydride. An homogeneous reaction mixture was obtained with this solvent and a shorter reaction time of 20 hours was employed.

For the determination, as acetic acid, of the α-hydroxyethyl groups present in lithium aluminum hydride reduction products, the procedure of Lemieux and Purves (80) was employed. For this analysis, the sample was oxidized in 30 percent aqueous chromium trioxide and the acetic acid formed was distilled and titrated. A provision is included in the method for the chromic acid which may pass into the distillate. Sucrose octaacetate (m.p. 85°-89°C., $[\alpha]_D^{23}$ +60.5°) was analyzed by this procedure and gave an equivalent weight of 85.4 grams. The theoretical value is 84.8 grams. This represents a recovery of acetic acid of better than 99 percent.

The results of the reduction of sucrose tosylate and of sucrose monomyristate tosylate are given in Table X.

8. The Preparation of Radioactive Sucrose Palmitates and the Chromatographic Separation of a Mixture of Sucrose Acetates.

During the course of this work, two samples of radioactive sucrose palmitates were prepared at the request of the Sugar Research Foundation for use by Dr. Quastel of McGill University. His studies of the metabolism of these esters have shown that the molecules are hydrolyzed in the stomach and consequently do not enter in the blood stream. Also, a sample of sucrose monoacetate was submitted to us for structural study by Dr. K.M. Herstein of the Herstein Laboratories of New York. The sample was described by the sender as a pure sucrose monoacetate. An account of this work is given below to preserve the continuity of the following section.

a. The Preparation of Radioactive Sucrose Monopalmitates.- The radioactive palmitic acid (27.9 mg. activity: 0.1 millicurie, C^{14} at carbon 1) was dissolved in 20 ml. of dry benzene and treated with an ether solution of diazomethane. The diazomethane was prepared from N-methyl N-nitroso urea by the method of Arndt (82). After the methylation, the solvents were removed and the radioactive methyl palmitate was transferred to a ten ml. volu metric flask as a benzene solution.

The two preparations were made according to the transesterification procedure given on page 31. For the first preparation, the radioactive methyl palmitate isolated from two ml. of the stock solution was diluted with five grams of cold methyl palmitate and transesterified for eleven hours. The nine gram yield isolated with n-butanol had an activity of two microcuries per gram. For the second preparation, eight ml. of the radioactive methyl palmitate were diluted with 1.275 grams of cold methyl ester. The radioactive ester isolated (1.925 g.) had a calculated activity of 40 microcuries per gram of ester.

b. The Analyses of a Mixture of Sucrose Acetates.- The sucrose acetate sent by Dr. Herstein was originally obtained from the reaction of one mole of sucrose with one mole of acetic anhydride in pyridine followed by precipitation of the product from n-butanol. This amorphous solid melted in the range of 57° to 70°C. and had a rotation, $[\alpha]_D^{20}$ +54.2° (in water). The ester saponified at 260 grams but gave acetyl group equivalents of 382 and 383 grams when analyzed for the acetyl content. The theoretical value for the acetyl group equivalent of sucrose monoacetate is 384 grams. The discrepancy between the saponification equivalent and the acetyl content remains obscure. Estimation of the sucrose content by the anthrone method

gave values of 89.6 and 90.0 percent sucrose (theoretical for sucrose monoacetate is 89.2 percent). Reprecipitation from dry n-butanol did not change the material appreciably. Chromatography on paper using the n-butanol/water system showed the presence of four well defined spots; R_f values: 0.05, 0.10, 0.146 and 0.283. On the same paper, sucrose had an R_f value of 0.05. Aniline phosphate was used as spray reagent.

The material was subjected to extrusion partition chromatography on Celite using n-butanol/water as developing solvent. The column was sprayed with alkaline permanganate (75) to reveal only three bands which were eluted with ethanol. The recovery was 67 percent. The weights of the fractions and their acetyl contents are listed in Table IV.

Table IV

Separation of a Mixture of Sucrose Acetates on Celite.

Fraction	R_f Value	Weight in mg.	Acetyl Content in m.e.	Acetyl Groups per mole
1	0.05 (a)	32.0	0.024	0.26
2	0.10 (b) 0.146	135.4	0.298	0.83
3	0.283(c)	26.7	0 110	1.71
Total		194.1 (d)	0.432	0.84

(a). The fraction was sucrose with a trace of material with R_f value about half of that of sucrose.

(b). The material was contaminated with a small amount of sucrose.

(c). The material was contaminated with a small amount of fraction 2 together with a very small amount of a substance with a greater R_f value, presumably sucrose triacetate.

(d). Sixty g. of Celite were used to chromatograph 280 mg. of the sucrose acetate in a column 36 mm. in diameter.

These data show that the sucrose acetate studied was a mixture consisting of approximately 17 percent sucrose, 69 percent sucrose monoacetate, 14 percent sucrose diacetate and some sucrose triacetate. The fact that the sample analyzed as a monoacetate was entirely fortuitous. The Celite chromatogram together with the results of the paper chromatograms show clearly that the main component (approximately 69 percent) was a mixture of isomeric sucrose monoacetates.

III. DISCUSSION OF EXPERIMENTAL RESULTS.

At the beginning of this investigation in early May 1955, no reference could be found in the chemical literature relating to the esterification of sucrose by transesterification. Preliminary studies had been made, however, by F.D. Snell and coworkers.

The procedure for the acylation of sucrose by transesterification employed throughout this work (see p. 31) was based on a memorandum sent to us by L. Osipow (81) of the Snell group. This method, which employed aeration to remove the methanol byproduct, was modified in the laboratory to exclude the passage of air. The higher vacuum used in the procedure employed in this work was adequate to remove the methanol. Potassium carbonate had been found by the Snell group to be the best catalyst for this particular reaction. It was employed in this investigation to the exclusion of others. The DMF solvent was chosen for similar reasons.

The method of isolation of the crude myristate ester product, using n-butanol or methyl ethyl ketone and five percent aqueous sodium chloride to partition the reaction cake, was developed in this laboratory. It was later adopted by other workers (41).

Preparations of sucrose stearates, palmitates and myristates done in the early stages of this work have resulted in the latter being adopted exclusively for the remainder of the study. This choice was based on the fact that the sucrose myristates possessed a superior hydrophile-lypophile balance and consequently, the properties of the constituents (sucrose and myristic acid) are well masked.

1. Preparations of Sucrose Myristates by Transesterification.

The results of a number of preparations of sucrose myristates using the procedure given on page 31 are listed in Table V. The purification procedures for the samples listed are given in the footnotes to Table V.

The results presented in Table V confirm a variety of observations reported by Snell and coworkers (41). It is to be noted first of all, that the crude products from the shorter reaction times have the higher ester group contents. The products isolated after eight hours or more of reaction time all possessed approximately 1.1 myristoyl groups per sucrose residue. The acylation of sucrose by transesterification with methyl myristate therefore definitely goes through a diester concentration maximum

Table V.

Preparations of Sucrose Myristates by Transesterification.

Sample (a)	Methyl myristate mM (b)	Reaction time in hours	Saponification equiv.	Fatty acid equiv.	Myristoyl groups per molecule	Percent yield (c)
1	50	13	515	-	-	88
2	175	8	521	-	-	85
2a			520	528	1.1	-
3	175	11	520	528	1.1	72
3a			552	-	1.0	-
4	175	7	478	480	1.27	60
5	175	6.5	408	414	1.72	74
5a			350	-	2.44	-
5b			528	534	1.08	-
6	58.3	11.5	512	519	-	90
6a			507	513	1.13	-

(a). The samples were isolated and purified as follows.

1. Isolated using methyl ethyl ketone.

2. Isolated using methyl ethyl ketone.

2a. Sample 2 (76 g.) was dissolved in 220 ml. of DMF-water (10:1) and extracted continuously with hexane for 24 hours. Evaporation of the DMF phase yielded this product. Paper chromatograms developed with n-butanol/water and sprayed with aniline phosphate sho-

Footnotes to Table V.

wed traces of sucrose to be present.

3. Isolated using methyl ethyl ketone and purified as described above for sample 2a.

3a. Sample 2a and 3 were combined (90 g.), dissolved in 350 ml. of n-butanol and extracted with five percent aqueous sodium chloride to remove the traces of sucrose. The butanol phase yielded a solid which was precipitated twice from 400 ml. of acetone to give 57 g. of product, ($[\alpha]_D^{23}$ +42.6° (c. 1 in methanol), softening point, 65°-70° C.). The amorphous solid was obtained as microspherical granules. This ester contained 57.5 percent sucrose as determined with anthrone (value for monoester is 62.0 percent sucrose).

4. Volatile amine, 28 m.e., was detected in the trap. The 73.6 g. of crude ester isolated using n-butanol, was dissolved in 350 ml. of DMF. When this solution was saturated with hexane, 1.5 g. of potassium soap precipitated. After filtration, 35 ml. of water were added to the DMF and the solution was extracted continuously with hexane for 21 hours. This removed 9.75 g. of highly substituted sucrose myristates (fatty acid equivalent of 357 g.). Evaporation of the DMF gave 62 g. of product which was precipitated from

Footnotes to Table V.

acetone to give 50 g. of this product.

5. Volatile amine, 23 m.e. was detected in the trap. Before removing the DMF, the reaction mixture was extracted five times with 200 ml. of hexane. This treatment removed 1.3 g. of solid. The 66.1 g. of crude ester product isolated using methyl ethyl ketone, was precipitated from 300 ml. of acetone to give 53 g. of this product.

5a. Sample 5 (25 g.) was precipitated from 125 ml. of methanol to yield 12.71 g. of this material.

5b. The methanol mother liquors from the preparation of sample 5a yielded 11.27 g. of solid which was precipitated from 50 ml. of acetone to yield this material.

6. Isolated using n-butanol.

6a. A five transfer counter-current distribution was done on 10 g. of sample 6 with the solvent system DMF: water: diethyl ether (1:1:2). Three tubes were used and 100 ml. of each phase was employed per tube. The combined bottom phases of tubes 0, 1 and 2 gave upon evaporation in vacuo at 50° C., 7.5 g. of product which afforded 6.17 g. of white solid after precipitation from 45 ml. of acetone. Paper chromatograms showed that traces of sucrose were present. These

Footnotes to Table V.

traces were removed by extracting a n-butanol solution of the ester with five percent salt solution. Evaporation of the butanol gave this sample.

(b). In each preparation, there was a two molar excess of sucrose. The potassium carbonate (0.1 molar level based on the methyl myristate) and the DMF (3.3 ml. per g. of sucrose) were used in the same relative amounts throughout.

(c). The yields are based on the methyl myristate and calculated using the analyses of the products by saponification.

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during the first seven hours of the reaction with thermodynamic equilibrium being reached only after about eight hours of reaction time. Such a course for the esterification can only be due to the initially formed monoesters undergoing esterification at a much greater rate than sucrose.

The preparation of samples 4 and 5 show that appreciable amounts of dimethylamine are formed. This would presuppose side reactions occurring with the solvent. In view of the need of the sucrose ester detergents in the pharmaceutical and food industries, the presence of the

toxic formate group in these products would have been a serious objection to the manufacturing process employing DMF as solvent. Such formate esters could result from the reaction of sucrose with DMF according to the following equation.

$$R\text{-}OH + HCO\text{-}N(CH_3)_2 \overset{K_2CO_3}{\rightleftharpoons} HCO\text{-}OR + HN(CH_3)_2$$

Although the agreement between the sapinification equivalents and the fatty acid equivalents of the esters eliminated the possibility of significant levels of the formyl group, one must remember that an analytical method is only as good as what is looked for.

The results of the determinations for formyl group carried out on the sucrose myristates employing the analytical method developed during this study (p. 35), have definitely shown that, under the conditions of the transesterification described on page 31, reaction times of 15 hours gave sucrose myristate samples containing less than 0.1 percent by weight of formyl group. This can be seen from the fact that 913.4 milligrams of sucrose myristate containing 1.24 myristoyl groups per sucrose molecule contained 0.0216 millimoles of formyl residue. Other side reactions possibly undergone by the solvent were not investigated.

The reports of Snell (41) concerning the presence of potassium soap in the sucrose fatty esters were confirmed during the present work. The 1.5 grams of soap isolated in the preparation of sample 4 represents 3.2 percent of the methyl myristate originally used. Other determinations have shown that after 15 hours of reaction time, 4.7 percent of the methyl ester had been converted to soap. The potassium soap isolated was analyzed by doing fatty acid contents and weighing the combustion residues. It was not determined whether this soap was formed during the esterification or during the isolation.

It was first attempted to separate the polyesters from the monoesters by extraction of the reaction mixture with hexane. The preparation of sample 5 showed this to be not effective. The preparation of sample 4 shows that the extraction of a DMF-water (10:1) solution of the crude ester product can be used to remove the tri- and higher esters.

A solution to the problem of separating mono- from diesters was provided by counter-current distribution. Preliminary studies with the solvent system DMF: water:diethyl ether (1:1:2) had shown that the di- and higher sucrose myristates were removed rapidly by the

successive ether extractions while the monomyristates remained in the bottom layers of the first three tubes. Thus, a counter-current distribution of one gram of sam ple 5 (originally containing 1.72 myristoyl groups per molecule of sucrose) using 50 ml. of each phase gave a polyester fraction which contained 2.76 groups per sucrose residue. Evaporation of the first three bottom layers gave a solid which appeared to possess 1.05 acyl groups when analyzed by saponification but had a fatty acid equivalent indicating only 0.91 groups. The discrepancy between the saponification and the fatty acid equivalents experienced with the products isolated from the bottom layers of these distributions is probably related to the ester molecules undergoing chemical change during the extraction and isolation processes. Paper chromatography showed that sucrose was liberated during these distributions. Some hydrolysis of the esters therefore occurred. The results obtained in the preparation of sample 6 show that pure sucrose esters were obtained when these fractions were precipitated from acetone.

Two solvents were found to be useful for the purification of sucrose myristates by precipitation procedures. The preparation of sample 5a shows that precipitation from methanol will remove the monoesters. On the

other hand, precipitation from acetone as in the preparation of sample 3a resulted in an enrichment in sucrose monomyristates. These acetone purifications were carried out by dissolving the ester in boiling acetone, filtering the hot solution and cooling the filtrate to bring about precipitation of the monoesters. That the procedure was accompanied by a small amount of conversion of sucrose monoesters into diesters and sucrose was indicated by the precipitation of sucrose while dissolving the sucrose free esters in the hot acetone. This conversion did not always occur and perhaps is related to traces of alkalinity.

2. Preparations of Sucrose Myristates Using the Acid Chloride.

Two preparations of sucrose myristates were made using the procedure described on page 33. The results are presented in Table VI.

The results given in Table VI show clearly that, as in the preparation of sucrose myristates by transesterification, the sucrose monomyristates formed by the action of myristoyl chloride in pyridine are esterified more rapidly than is sucrose. Similar results have been observed when sucrose was acetylated with acetic anhydride in pyridine (85). The acylation in pyridine is irreversible

Table VI

Preparations of Sucrose Myristates using the Acid Chloride

Sample (a)	Sucrose mM	Myristoyl chloride mM	Saponification equiv.	Fatty acid equiv.	Myristoyl groups per molecule	Percent yield (b)
7	73.2	36.6	384	391	1.96	93
7a			311	-	3.39	-
7b			539	-	1.04	-
8	109.8	37.6	365	365	2.21	92
8a			320	333	2.77	-
8b			558	534	1.05	-

(a). The samples were isolated and purified as follows.

7. Isolated as described on page 33, the substance gave a negative Fehling's test.

7a. Sample 7 (10 g.) was precipitated from 65 ml. of a mixture of ethanol and methanol (1:5). The yield was 4.35 g. The material contained no free acid and was soluble in hexane.

7b. The mother liquors from the preparation of sample 7a yielded 4.89 g. of solid which was precipitated from 70 ml. of acetone. The yield was 2.79 g.

8. The material, isolated as described on page 33, was free of sucrose and gave a negative Fehling's test.

8a. A five transfer counter-current distribution was made

Footnotes to Table VI.

with 10 g. of sample 8. The solvent system was DMF:water:diethyl ether (1:1:2). Three tubes were used and 200 ml. of each phase was employed per tube. The extractions had to be centrifuged to break the emulsions. The combined top phases of tubes 5, 4 and 3 gave 7.0 g. of this sucrose free material.

8b. Concentration of the bottom phases of tubes 0, 1 and 2 from above gave 3.0 g. of a dark residue shown by paper chromatography to contain sucrose. This solid was treated with 20 ml. of boiling acetone, filtered and a 1.56 g. crop of white solid was obtained on cooling the filtrate. This solid was dissolved in n-butanol and extracted with five percent salt solution to remove the traces of sucrose still present. The material was recovered by evaporation of the butanol.

(b). The yields are based on the myristoyl chloride and were calculated using the myristoyl content determined by saponification.

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and, in contrast with the transesterification, the first products of the reaction are those isolated.

It is noteworthy that the purification of sample

8b resulted in extensive darkening and hydrolysis. This sample, which analyzed at 1.05 myristoyl groups per molecule of sucrose is therefore probably not representative of the original monomyristate sample.

3. The Rate of the Transesterification Reaction.

Snell and coworkers (41) have done rough reaction kinetics of the preparation of fatty esters of sucrose by transesterification. They reported that, under reaction conditions which compare closely to those employed during this work (see p. 15), the methyl fatty ester had completely reacted after four to six hours of reaction time. The aliquots withdrawn from the reaction mixtures were dried under reduced pressure at 100° C. before being analyzed. This isolation step undoubtedly changed the composition of the sample withdrawn from the reaction mixture. It was therefore decided to transfer aliquots of the reaction mixture directly to columns for chromatographic analysis. The results plotted in Fig. 2 indicate that the transesterification of methyl myristate with sucrose was complete in seven to eight hours of reaction time. This result is in agreement with the rates suggested by the data reported in Table V. It was not possible to continue studies along this line and the reason for the low recoveries of the

sucrose remains obscure. It is felt that this approach for the study of the kinetics of the transesterification reaction warrants further attention.

4. The Structure of the Sucrose Esters.

In view of our inability to separate the sucrose monomyristates into the positional isomers, these com pounds were examined as mixtures.

a. Determination of the Number of Myristoyl Groups Occupying the 6- and 6'- Positions in a Sucrose Myristate.- The results obtained on treatment of the tosyl esters of sucrose and sucrose derivatives with sodium iodide in acetone are listed in Table VII. The compounds tosylated are those reported, using the same numbers, in Tables V and VI. Figure 3 is a plot of the iodine atoms introduced into a tosylated sucrose monomyristate (Table VII, compound 3a) after a variety of reaction times. Similar results obtained with tosylated sucrose (Table VII) are included.

A kinetic study of the iodination reaction (Fig. 3) showed that all the replaceable tosyloxy groups had reacted after eight hours. Reaction times longer than 15 hours resulted in secondary reactions taking place as in-

Table VII.

Tosylation and Iodination Experiments.

Compound	Myristoyl groups per molecule	Tosylation			Iodination				Myristoyl groups at 6- and 6'-
		Percent sulfur	Percent yield (a)	Groups introduced	Reaction time, hrs.	Percent iodine	Percent yield (a)	Atoms introduced	
Sucrose	-	14.93	91	5.67	2	19.55	85	1.75	-
"	-	"	"	"	13	22.55	94	2.00	-
Methyl -D-gluco-pyranoside (b)	-	14.29	98	2.76	2	25.62	72	1.15	-
"	-	"	"	"	13	28.28	-	1.25	-
2,3,6,3',4'-penta-O-acetyl sucrose (c)	-	9.35	-	2.93	7	13.40	97	1.02	-
Sucrose myristates:									
3a	1.00	12.33	84	5.24	2	11.72	94	1.21	-
"	"	"	"	"	4	14.11	87	1.44	-
"	"	"	"	"	8	14.82	-	1.51	-
"	"	"	"	"	13	15.03	89	1.53	0.47
4	1.27	11.23	88	4.65	8	12.91	-	1.29	0.71

Table VII (continued).

Compound	Myristoyl groups per molecule	Tosylation			Iodination				Myristoyl groups at 6- and 6'-
		Percent sulfur	Percent yield (a)	Groups introduced	Reaction time, hrs.	Percent iodine	Percent yield (a)	Atoms introduced	
Sucrose myristates:									
5a	2.44	8.66	-	3.96	8	8.35	93	0.94	1.06
5b	1.08	11.90	86	4.93	8	15.22	97	1.52	0.48
6a	1.13	12.00	80	5.14	8	14.48	93	1.49	0.51
7a	3.39	5.95	85	2.74	8	4.99	82	0.57	1.43
7b	1.04	11.45	80	4.45	7	13.86	89	1.30	0.70
8a	2.77	6.91	65	2.98	8	5.70	73	0.61	1.39
8b	1.05	11.92	68	4.91	8	16.89	80	1.66	0.44

(a). The yields were based on the theoretical amounts expected from the analyses.

(b). Melting point, 165.5°C., rotation, $[\alpha]_D^{23}$ +158° (c. 8 in water).

(c). Kindly provided by Mr. J.P. Barrette.

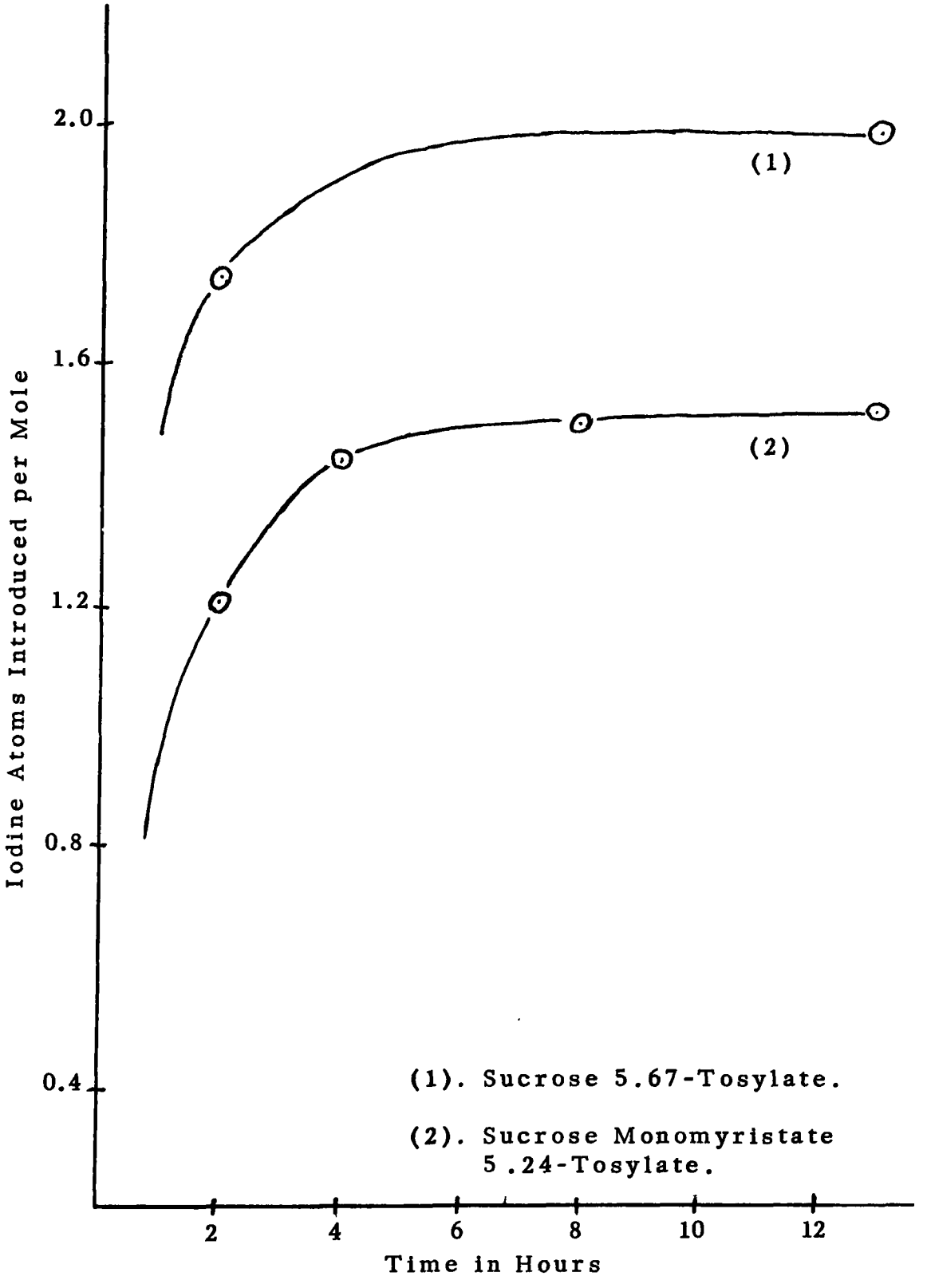

Fig. 3. The Iodination of the Tosylates of Sucrose and of Sucrose Monomyristate.

dicated by the liberation of more and more iodine. The iodinations were therefore stopped after eight hours.

It is well established (56, 57) that primary tosyloxy groups are usually readily replaced by iodine when the compound is treated with sodium iodide in acetone. Thus, 1.25 tosyloxy groups of a methyl α-D-glucopyranoside 2.76-tosylate underwent replacement. This high result was probably due to elimination of secondary tosyl groups in the course of the reaction. Certainly, the results indicate that one of the tosyloxy groups was more readily replaced than the others. Iodination of the sucrose 5.67-tosylate led to the replacement of two of the tosyloxy groups. This fact together with the fact that 4,1',6'-tri-O-tosyl sucrose pentaacetate underwent replacement of only one of the tosyloxy groups shows that the 6-tosyloxy group of a sucrose tosylate is readily replaced by iodine. Also, the results show that either the 1'- or 6'-tosyloxy group of a sucrose tosylate is replaceable by iodine. That the replaceable tosyloxy group is that situated at the 6'- position is clearly inferred by the steric requirements for the iodination reaction. It is well established that these reactions are of the S_N2 type and proceed by way of an attack at the rear side of the carbon carrying the tosyloxy group. Ingold and coworkers (83) have shown that such an

attack is strongly prohibited in the case of neopentyl halides. The situation at the 1' position of a sucrose tosylate closely resembles that in a neopentyl halide and, consequently can be expected to be inactive for similar reasons. That this is in fact the case is supported by the observation that 2,3;4,5-di-O-isopropylidene-β-D-fructose tosylate is stable to iodination (84).

On the basis that only the 6- and 6'- tosyloxy groups in a sucrose tosylate are replaceable by iodine, the iodinations of the tosylates of samples 3a, 5b, and 6a establish that approximately half of the acyl groups of these sucrose monomyristates are at the 6- and 6'- positions. Re-interpretation of the results obtained by Snell and coworkers (61) (p. 27) indicates that only 37 percent of the acyl groups of the sucrose monolaurate studied were at the 6- and 6'- positions.

The tosylation and iodination experiments performed on the sucrose monomyristate obtained by acylation with myristoyl chloride (sample 7b, Table VII) suggest that 70 percent of the myristoyl groups were at the 6 and 6'- positions. Thus, on the basis of this experiment, it appears that the monoester prepared by acylation possesses approximately 0.2 more myristoyl groups at the 6- and

6'- positions. This is reasonable since these primary positions can be expected to undergo acylation more rapidly than the other more hindered positions. However, the results with sample 8b (Table VII) throw some doubt on this conclusion. It is to be noted, however, that the preparation of sample 8b was accompanied by extensive hydrolysis in the course of the counter-current distribution. Thus it may prove that myristoyl groups at the 6- and 6'- positions are more susceptible to hydrolysis than those at the other positions.

b. Periodate Oxidations.- The periodate oxidation of a sucrose derivative can be used to obtain information regarding the nature of the positional isomer or isomers present in the derivative. This is seen from Table VIII which gives the theoretical values for the consumption of periodate and the production of formic acid when the variety of possible mono-O-substituted sucroses are oxidized by the reagent.

The oxidation of 72.88 milligrams of pure sucrose monomyristate prepared by transesterification (sample 3a, Table V) resulted in 2.87 millimoles of periodate being consumed after two days. The results of this oxidation are given in Table IX.

Table VIII

The Consumption of Periodate and the Production of Formic Acid for the Possible Mono-O-Substituted Sucroses.

Position of Substituent group	Molar equivalent of periodate consumed	Molar equivalent of formic acid produced
6,1' or 6'	3	1
2 or 4	2	0
3	1	0
3' or 4'	2	1

Table IX

Periodate Oxidation of Sucrose Monomyristate at 24.8°C.

Reaction time, hours.	mM of sodium periodate per mM of sucrose myristate.
0.3	0.36
0.5	0.89
3	1.43
4	1.66
24	2.08
48	2.87

The rate of uptake of oxidant was slower for the sucrose esters than for sucrose because part of the ester

was dispersed as a fine colloidal suspension in the oxidation media. Extensive hydrolysis of the ester linkage was ruled out, since after three days, the reaction mixture still had the properties of a detergent solution and the fine amorphous precipitate which had formed could be readily dissolved in aqueous methanol with foaming. Apparently, part of the oxidation product had precipitated out of solution.

Attempts to oxidize sucrose monomyristate samples in 50 percent aqueous methanol, in order to increase the solubility of the esters in the reaction media, have resulted in consumptions of periodate exceeding three millimoles per millimole of ester. Thus, sample 5b (Table V), which contained 1.08 myristoyl groups per molecule, consumed 3.19 millimoles of oxidant after two days. This result, which must be due to partial hydrolysis of the glycosidic linkages, casts some uncertainty on the value of the method for studying the structure of sucrose esters. Nevertheless, the results support the conclusion reached by Snell and coworkers (61) that the monoesters obtained by transesterification are substituted almost entirely at the primary positions of sucrose. This structure for a sucrose monoester prepared by transesterification would not be surprising since it is well established (86)

that acyl groups have a strong tendency to migrate to primary positions especially in alkaline media as is used in the transesterification.

In view of the above results, it can be concluded with a certain degree of certainty that the myristoyl groups of the sucrose monomyristate are substantially entirely situated at the three primary positions. Since approximately one-half of the groups were shown to be at the 6- and 6' positions by the tosylation and iodination method of analysis, it follows that the other half of the groups are at the 1'- position.

c. The Reduction of Tosyl Esters.- Karrer and coworkers (79, 87) have shown that in some cases, the lithium aluminum hydride reduction of aliphatic primary tosyloxy groups results in the replacement of the tosyloxy group by hydrogen. On the other hand, the reduction of a secondary tosyloxy group can be expected to undergo reduction to the hydroxyl group. If it could be shown that the three primary tosyloxy groups of a sucrose tosylate are reduced to terminal methyl groups by lithium aluminum hydride, then a means of estimating the number of tosyl groups at the 1'- position would be available since the number of tosyloxy groups at the 6- and 6'- po-

sitions can be determined by iodination. The results of the reductions of sucrose tosylate and of sucrose monomyristate tosylate are given in Table X.

Table X

Reduction of the Tosylates of Sucrose and of Sucrose Monomyristate with Lithium Aluminum Hydride.

Tosylate	Solvent	Terminal C-methyl group equivalent	Percent yield (a)
1. Sucrose 5.67-tosylate	diethyl ether	107.8 g.	38
2. "	tetrahydrofuran	106.0 g.	38
3. Sucrose monomyristate 5.24-tosylate	diethyl ether	198.1 g.	85

(a). The yields are based on the analyses of the products. It was assumed that the tosyloxy groups not reduced to terminal C-methyl groups were reduced to hydroxyl functions. The reduction products were chromatographed on paper using the n-butanol/water system and the spots were located using the permanganate-periodate spray reagent. Only a few of the compounds detected by this reagent (those with low R_f values) were sensitive to the aniline phosphate spray reagent. The results of these chromatograms are given below.

1. The reduction of this tosylate (see Table VII) gave four main products having R_f values of 0.22, 0.36, 0.45 and 0.65. Treatment of this material with 4 N hydrochloric acid at 80 C. for 15 minutes did not change the chromatographic separation.
2. This material on paper chromatograms appeared to be the same as that obtained in the reduction with diethyl ether.
3. The reduction of this tosylate (sample 3a, Table VII) gave a mixture of six main components having R_f values of 0.074, 0.135, 0.235, 0.34, 0.475 and 0.662. The slower component had an R_f value identical to that of glucose and could be detected by the aniline phosphate spray reagent.

All the reduction products were sulfur free colourless glasses. At least some of the components possessed rather high vapour pressures since losses were obtained in attempts to dry the substances under vacuum.

Examination of Table X is sufficient to show that the reaction is very complex. It is interesting to note that the reduction of sucrose 5.67-tosylate under two different conditions gave identical yields of products which analyzed very closely to trideoxysucrose (C-methyl group

equivalent for 6,1',6'-trideoxysucrose is 98.1 g.). The analysis of the product obtained on the reduction of the sucrose monomyristate tosylate (sample 3, Table X) suggests that 1.6 terminal methyl groups were present. The theoretical terminal C-methyl group equivalent for a dideoxysucrose containing two C-methyl groups is 155.15 grams.

The fact that the chromatographic pattern of these products was not changed by treatment with mineral acid, plus the fact that the reduction of the sucrose myristate tosylate gave a component in the product mixture behaving like glucose on paper chromatograms, tend to show that the glycosidic bonds were broken during the course of the isolation of the reduction products. That this is the case was indicated by the fact that the reduction of sucrose octaacetate under identical conditions led to the isolation of glucose and fructose.

In view of the complexity of the reduction products and the low yields obtained, the results are not amenable to interpretation.

CLAIMS TO ORIGINAL RESEARCH

1. The 1'- tosyloxy group of a sucrose tosylate is not replaceable by an iodine atom.
2. A method was established for estimating the percentage substitution at the 6- and 6'- positions of a sucrose derivative.
3. A method was developed to estimate the formyl group content of a sucrose ester.
4. Radioactive sucrose palmitate having C^{14} at the C 1 position of the palmitoyl group was prepared.
5. Sucrose monomyristates were separated from sucrose dimyristates by counter-current distribution.
6. Sucrose was separated from sucrose myristates by extraction using n-butanol and five percent aqueous sodium chloride.
7. Proof was obtained that the sucrose monomyristates obtained by transesterification have approximately 50 percent of the acyl groups on the 6- and 6'- positions.
8. A modification of the aniline phosphate spray reagent of Bryson and Mitchell (68).
9. A mixture of sucrose, sucrose monoacetates and sucrose diacetates was separated by partition chromatography both on paper and on Celite.

10. The acylation of sucrose with myristoyl chloride in pyridine gives a mixture of highly substituted sucrose myristates.
11. The lithium aluminum hydride reduction of sucrose 5.67-tosylate gave a mixture of four compounds.
12. The lithium aluminum hydride reduction of sucrose monomyristate 5.24-tosylate prepared by transesterification gave a mixture of at least six compounds.

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