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THE PREPARATION AND STRUCTURE OF SUCROSE MONOESTERS

(PART ONE)

and

THE ESTERIFICATION OF 1,4;3,6-DIANHYDRO-D-GLUCITOL

(PART TWO)

Thesis submitted by

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In partial fulfillment of the requirements for the

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PREFACE

Considering the large annual production, high state of purity and the economic importance of sucrose it is surprising that, until recently, the compound has been subjected to very few organic chemical investigations other than efforts to establish its structure. Part I of this thesis deals with a systematic kinetic study of the transesterification of sucrose with the methyl ester of a fatty acid in N,N-dimethylformamide solution (using a base as catalyst), and the major equilibrium reactions which occur during the formation of sucrose monoester. The conclusions reached by previous workers on this subject were speculative or unwarranted and left the impression that the transesterification reaction and the structure of sucrose monoester merited more accurate delineation. The purpose of our investigations was to develop a satisfactory process for the preparation of sucrose monoester, and to elucidate the structure of the latter. This thesis describes and interprets the relevant experimental data which enabled these objectives to be fulfilled.

Part II of this thesis unambiguously establishes the structures of the isomeric mono-O-tosyl mono-O-acetyl

derivatives of 1,4;3,6-dianhydro-D-glucitol, and provides evidence that the endo-5-hydroxyl group is acylated, in pyridine, more rapidly than the exo-2-hydroxyl group.

These investigations were supported by a grant made to Professor R.U. Lemieux by the Sugar Research Foundation Inc., New York.

I have been aided in many ways during the course of this research by Dr. C.T. Bishop of the National Research Council, and I wish to acknowledge this assistance. I should also like to thank the staff of the Department of Chemistry of the University of Ottawa, and in particular Professors F.A.L. Anet and R.R. Fraser, for their helpful suggestions and stimulating discussions. Above all I would like to express my gratitude to Professor R.U. Lemieux whose scientific judgement has been the mainstay of this investigation.

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THE PREPARATION AND STRUCTURE OF SUCROSE MONOESTERS

(PART ONE)

ABSTRACT

The preparation of sucrose monoester by the transesterification of sucrose with the methyl ester of a fatty acid, in N,N-dimethylformamide solution, using solid potassium carbonate as catalyst has been reported previously (1,2,3,4). All of these preparative methods, however, were designed on a purely empirical basis, and did not give a reproducible product in high yield. Furthermore, many conflicting conclusions were drawn, from insufficient experimental evidence, about the mechanism of the reaction (3,4) and the effect of reaction variables on the product composition (2,3,4). The present work reports the results of a systematic physical chemical investigation of this reaction, and the structure of sucrose monoester.

The standard reaction mixture was maintained at 80° and 75 mm pressure, and was 18.75, 6.25 and 1.25 x 10^{-2} molar in sucrose, methyl ester and sucrate ion, respectively, and N,N-dimethylformamide was used as solvent. The removal of methanol from the reaction solution was facilitated by passing a dry stream of nitrogen through the reaction solution. All rates of reaction were determined by following the decrease in concentration of methyl ester, by gas-liquid partition chromatography, using n-octadecane as an internal standard. Methyl myristate was used for most of the kinetic studies.

It was not possible to reproduce rates of reaction or yields of sucrose monoester when solid potassium carbonate was used as catalyst. This was attributed to variations in the rate at which potassium carbonate dissolved in the reaction mixture. When methanolic solutions of potassium carbonate were used, the reaction proceeded in homogeneous solution, and gave reproducible rates of reaction and yields of sucrose ester. The rate of reaction was independent of the base used as catalyst. For example, equivalent amounts of potassium carbonate and potassium methoxide gave the same rates of reaction. Saturated aqueous solutions of potassium carbonate and potassium hydroxide, as well as saturated methanolic solutions of potassium hydroxide, gave inferior rates of reaction and yields of sucrose ester. The nature of the cation had little effect on the rate of reaction. In fact, the rates of reaction using equivalent amounts of lithium, sodium and potassium methoxides were essentially the same. It was also found that the rate of transesterification was independent of sucrose concentration and the chain length of the fatty acid (C_{14}, C_{16} and C_{18}) in the methyl ester. Furthermore, since the use of a stream of carbon dioxide, instead of nitrogen strongly depressed the rate of reaction, when potassium carbonate was used, the effective catalyst for the reaction was shown to be sucrate ion. The rate of reaction was found to follow first order

kinetics in sucrate ion, at concentrations less than 3.13 x 10^{-3} moles per liter, and in methyl ester. At higher concentrations of sucrate ion the reaction behaved in an non-ideal manner until the point of saturation was reached at 12 x 10^{-3} moles per liter. The standard trans-esterification mixture (see above) gave approximately 92 percent reaction and sucrose ester in approximately 82 percent yield in a reaction time of 150 minutes. This is the shortest reaction period ever reported for this reaction at 80°. The rate of reaction doubled for a 17° increase in temperature, and the activation energy and frequency factor for the reaction was 9.9 kcal $mole^{-1}$ and 0.7 x 10^{5} liters $moles^{-1}$ sec^{-1}, respectively. The thermodynamic constants for the reaction were

$$\Delta F^{\ddagger} = 22 \text{ kcal mole}^{-1},$$
$$\Delta H^{\ddagger} = 9 \text{ kcal mole}^{-1},$$
$$\Delta S^{\ddagger} = -38 \text{ entropy units.}$$

The equilibrium constant for the reaction,

Sucrose + Methyl myristate $\rightleftharpoons$ Sucrose myristates + Methanol,

was found to be 0.57, when the equilibrium condition was approached from both sides. This value established the necessity for removing methanol from the reaction solution if sucrose ester was to be made in high yield. The equilibrium constant for the reaction,

Sucrose + Sucrose dimyristate $\rightleftharpoons$ 2 Sucrose monomyristate

was found to be 1.9 $\pm$ 0.1 at two different concentrations of sucrose. Using C^{14}-labelled sucrose, it was established that the rate of the exchange of sucrose with sucrose esters was too rapid to expect any product control through kinetic control. In other words, at any time throughout the transesterification only the thermodynamic product of the reaction prevails.

Finally, the structure of sucrose monomyristate was established by the use of gas-liquid partition chromatography and nuclear magnetic resonance on appropriate derivatives. Sucrose monomyristate was found to be a mixture of 6'-myristoyl sucrose, 6-myristoyl sucrose and unidentified esters in the relative proportions of 0.62 to 0.28 to 0.10, respectively. This is the first time that the structure of a disaccharide has been determined by way of an application of nuclear magnetic resonance.

INTRODUCTION

The fatty acid monoesters of sucrose are of potential economic importance as low cost detergents. Furthermore, they could be used widely in the food, cosmetic and pharmaceutical industries since they are tasteless, do not irritate the skin or eyes, and are non-toxic. Sucrose monoesters containing an unsaturated fatty acid also have drying-oil properties. However, since the economic prospects, uses and industrial applications of sucrose esters have already been reviewed in detail (5,6,7), only those subjects relating to their preparation will be discussed.

1. Sucrose (α-D-glucopyranosyl-β-D-fructofuranoside)

Sucrose, known colloquially as table sugar, is the most abundant and widespread carbohydrate found in the vegetable kingdom and is of great economic importance as a sweetening substance and a food. The combined annual production of raw sugar, from sugar cane and sugar beet, reached forty-seven million tons in the year 1957-58. Furthermore, since raw sugar is made to a purity of 96 percent and refined sugar is at least 99.9 percent pure, this is by far the largest amount of any pure organic chemical made in any industry. In spite of its abundance, however, this pure highly crystalline organic compound has

been the subject of relatively few published organic chemical investigations apart from work relating to its isolation and purification, and the proof of its structure and synthesis.

Sucrose is a sweet, crystalline solid which melts at 188° and has a specific rotation $[\alpha]_D$ + 66.53°. Hydrolysis of sucrose with an acid or enzyme gives equimolar amounts of D-glucose and D-fructose and is a classical example of a bimolecular pseudo first order reaction (8). Sucrose does not reduce Fehling's solution or react with phenylhydrazine.

Work on the structure and configuration of sucrose up until 1949 had shown that D-glucose and D-fructose were linked through the anomeric carbon atoms and were present as a six-membered "pyranose" ring and five-membered "furanose"ring, respectively (9). Furthermore, the presence of an α-configuration of the D-glucose moiety was indicated when the enzyme "invertase", a β-fructofuranosidase, cleaved the bond between the anomeric carbon and oxygen of fructose (10) and gave α-D-glucose (8) as the initial product of hydrolysis. Purely chemical evidence for the α-glucosidic linkage in sucrose was obtained when Lemieux and co-workers synthesized three known α-D-glucopyranosides, maltose (11), trehalose (12) and isomaltose (13) using Brigl's anhydride and then used the latter in the synthesis of sucrose (14).

A rationalization of the properties of Brigl's anhydride in these syntheses enabled Lemieux and Huber to conclude that there was an α-D-configuration of the glucosidic linkage in sucrose (14). Furthermore, the specific hydrolysis of the more levorotary of two methyl fructofuranosides by the enzyme "invertase" (15) indicated, on the basis of Hudson's isorotation rules (16), that glycosides which were unstable to the enzyme, such as α-D-glucopyranosyl-D-fructofuranoside, had a β-configuration. Although the configuration of the anomeric centers of sucrose were established by x-ray crystallographic analysis (17) in 1947, it was not until recently that the β-configuration of the D-fructose moiety was established chemically by the formation of 1,4;3,6-dianhydro-β-D-fructofuranosyl 3,6-anhydro-α-D-glucopyranoside (18).

Although the conformation of sucrose in the crystalline state is known (17) there is no evidence available at present which establishes that the conformation in solution is exactly the same. The conformational features of α-D-glucopyranosyl-β-D-fructofuranoside, shown in the following diagram (see Figure 1), are those which could reasonably be expected for a molecule possessing a minimum of non-bonded interactions, and which are supported by the x-ray analysis of the compound in the crystalline state (17).

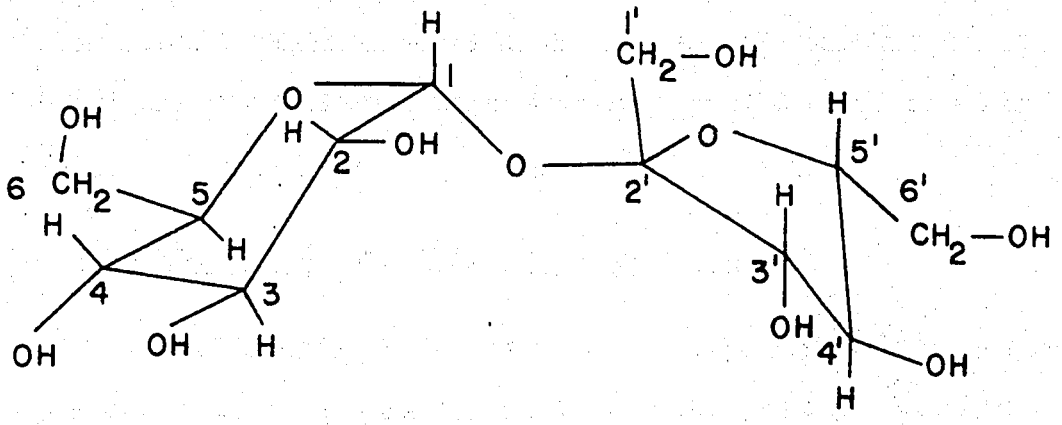


Figure I. Sucrose, α-D-glucopyranosyl-β-D-fructofuranoside.

The system of numbering the carbon atoms in the sucrose molecule is the same as that used by Hockett and Zief (19). The D-glucose moiety is numbered with numerals and the D-fructose moiety with prime numerals (see Figure I). Although some work has been done on the relative reactivities of hydroxyl groups in some carbohydrates (20), little is known about the relative reactivities of the hydroxyl groups of sucrose except that primary are more reactive than the secondary hydroxyl groups (21, 22), and that the primary hydroxyl groups at the 6- and 6'- positions appear to be more reactive than at the 1'- position (22) when a product of kinetic control is obtained. There is no

information available in the literature on what thermodynamic products would be formed when a reaction is carried out under equilibrating conditions except in the case of the acetyl migration from the 4 to the 6 position of sucrose (22).

2. The fatty acid monoesters of sucrose

Although the direct esterification of a polyhydric alcohol, such as glycerol, with a fatty acid is the oldest recorded method for the preparation of esters (23) it is impracticable because sucrose would caramelize at the high temperatures required for this type of reaction (24,25,26, 27).

The rapid inversion of sucrose with acid (8) also precludes esterification procedures which use an acid to catalyse the esterification reaction.

Acid chlorides (28,29,30) using a tertiary amine as an acid acceptor, or acid anhydrides (31,32,33), using the sodium salt of the acid as a buffering agent, may be used to esterify sucrose. The former process, however, is expensive (6) and is of little value in preparing high yields of mono- or even diesters, since the first products of reaction are esterified faster than sucrose itself (3,30). Furthermore, the use of acid anhydrides is only economical and practicable in the case of the lower fatty

acid anhydrides such as acetic and isobutyric anhydride (5,6).

The transesterification, or alcoholysis, reaction is the most satisfactory procedure for the preparation in high yield of fatty acid esters of sucrose (1,2,5,6). The reaction can be represented as

$$\text{Sucrose} + \text{Methyl ester of fatty acid} \underset{\text{catalyst}}{\rightleftharpoons} \text{Sucrose ester} + \text{Methanol}$$

Although the fatty acid ester of any alcohol can be used for the above reaction, the methyl ester is most suitable since the equilibrium can be displaced in favour of the forward reaction by removing the methanol from the reaction solution. The alcoholysis reaction is catalysed by both acid and base (34,35). The latter is preferred in the case of sucrose, which is stable to alkali, and no acidic substance is present which could neutralize the catalyst. Furthermore, superior rates of reaction are obtained in the alcoholysis reaction when a base is used to catalyse the reaction (34). Indeed the methyl esters of the fatty acids themselves are prepared, from animal or vegetable fats, by the transesterification reaction using a base as catalyst (34).

The method for preparing long chain fatty acid mono- and diesters of sucrose was developed by Snell in 1954. This involved the transesterification of sucrose

with the methyl ester of a fatty acid in N,N-dimethyl-formamide (DMF) using an inorganic base as catalyst. In the original publication (1), Snell described three different procedures for the preparation of sucrose esters which will be discussed separately below.

In the first procedure three moles of sucrose were reacted with one mole of methyl ester in four liters of DMF (technical grade) at 60° for three hours, using 0.2 moles of sodium methoxide as catalyst. At the end of the reaction period only 25 percent of the methyl ester had been converted to sucrose ester. A similar result was obtained when a glyceride ester was used in place of the methyl ester. During this work no attempt was made to enhance the rate of the reaction, or the yield of sucrose ester, by removing the methanol formed in the reaction.

The second procedure, and the preferred one, involved the reaction of three moles of sucrose with one mole of methyl ester in anhydrous DMF (3.3 ml of DMF per g of sucrose), at 90-95° and 80-100 mm pressure, using 0.1 moles of solid potassium carbonate as catalyst. The reaction mixture was stirred mechanically, and a six plate fractionating column was used to strip the methanol from the reaction solution. Aliquots of the reaction mixture were taken at intervals and analysed for sucrose, methyl ester, soap and sucrose mono- and diester (see below for

a description of the analytical procedures). It was claimed that the analytical data showed that the molar ratio of sucrose mono- to diester was 1:1, 2:1 and 23:1 after three, six and twelve hours, respectively. Snell concluded, therefore, that the sucrose monoesters, which were formed initially were esterified more rapidly than sucrose to form di- or polyesters of sucrose. The latter subsequently reacted with sucrose, on prolonged heating, to reform sucrose monoester. This conclusion was supported by the work of Billy who claimed that shorter reaction times produce a sucrose ester having a higher acyl content (3). Herstein and his coworkers also concur with the belief that sucrose diester is formed rapidly and then reacts with sucrose to form sucrose monoester (4,36). Snell also claimed that almost 20 percent of the potassium carbonate was converted to soap if the reaction was continued for twelve hours. Billy, on the other hand, states that only 5 percent soap is obtained in a reaction time of fifteen hours (3). A lower level of DMF (2.3 ml of DMF per g of sucrose) did not influence the initial rapid esterification of sucrose but had a pronounced effect on the subsequent conversion of sucrose diester, or polyester, to sucrose monoester (1). For example, the molar ratio of mono- to diester was 3.1:1 and 4.5:1 after seven and fourteen hours, respectively, whereas in the more dilute

solution (see above) the ratio was 2:1 and 23:1 after six and twelve hours, respectively. Furthermore, Snell found that the major effect of increasing the catalyst concentration was an increase in soap formation. This is in contrast to the results of Kononenko and Kastenbaum of the Herstein laboratories, who claim that the molar ratio of sucrose mono- to diester is dependent on the catalyst concentration (4). Dimethylsulphoxide was also used as a solvent in the transesterification reaction but was found to be less suitable than DMF (1,3). Although the actual weight yields of the sucrose ester, prepared in this manner (1), were not reported it was claimed that sucrose mono- or diester of 90 percent purity could be obtained.

In the third procedure (1) sucrose monoester was reacted with excess methyl ester, in the absence of a solvent, to form polyesters of sucrose which in turn were reacted with excess sucrose in the presence of a catalyst, to reform sucrose monoester. However, no experimental details of this method were reported.

In 1957 Snell reported that the rate of conversion of sucrose polyester to sucrose monoester and the rate of transesterification were dependent on the moisture content of the reaction solution (2). The former was claimed to be a maximum when the moisture content was

0.05 to 0.15 percent of the reaction solution. For example, when two equimolar reaction mixtures of sucrose and sucrose diester were equilibrated, in DMF containing 0.15 and 0.30 percent of water, respectively, at 90° and atmospheric pressure for three hours, the sucrose monoester content was 63.8 and 43.6 percent, respectively. The rate of the transesterification was found to decrease rapidly with increasing moisture content and became very slow when the latter was 2 percent of the reaction mixture. In an experiment carried out at 120° it was found that 0, 14.7 and 15.1 percent of the methyl ester was unreacted, and the sucrose monoester content of the reaction product was 73, 100 and 100 percent, after 1.5 hours when 0, 0.08 and 0.15 percent of water was present in the reaction mixture. Kononenko, however, claims to have shown that the relative proportions of sucrose mono- to diester were unaffected by the moisture content (4, 36).

The following routine analytical procedures were developed by Snell (1,2) to assay, rapidly, the products obtained from the transesterification reaction.

A sample of the crude sucrose ester was dried at 100° under reduced pressure, and then partitioned between n-butanol and 10 percent aqueous sodium chloride solution. The sucrose and percentage sucrose mono- and diester concentrations were calculated from the rotations

of the aqueous and n-butanol phases, respectively. Since a precise knowledge of the specific rotations of sucrose mono- and diester were required to estimate their relative concentrations, Snell (1) claimed that products were isolated which analysed for these compounds. Apart, however, from reporting that the specific rotations of these compounds in n-butanol were 39.1° and 25.6°, respectively, no detailed analytical data was supplied to support this claim. The soap present in the product was estimated by titrating an aliquot of the n-butanol phase with standard acid, using methyl orange as an indicator. Unreacted methyl ester was determined by saponifying the residue, from an aliquot of the n-butanol phase, with aqueous alkali. The latter was then distilled and the methanol, after oxidation with potassium permanganate, was determined as formaldehyde with chromatropic acid (37).

Two laboratory procedures were developed by Snell (1) to isolate and purify the fatty acid monoesters of sucrose. In the first method the DMF reaction mixture was reduced to one third of its original volume, by distillation under reduced pressure, and then extracted with eight, one liter, aliquots of n-hexane to remove any unreacted methyl ester. The DMF solution was then diluted with five volumes of acetone and the sucrose which precipitated was collected on a filter and washed with a

hot n-butanol solution. After removal of the solvent, acetone/n-butanol, under reduced pressure the residue was dried over sulfuric acid at 80°. The dry residue was then dissolved in acetone (10 g of sucrose ester per 200 ml of acetone) and cooled to -20° to precipitate the product. The latter was then dried, under reduced pressure, over sulfuric acid. The second procedure involved partitioning the crude product between n-butanol and 10 percent aqueous sodium chloride solution (3). After drying the n-butanol layer, with anhydrous sodium sulphate, a product was obtained containing sucrose ester, methyl ester and soap. The latter was then purified by precipitation from acetone as described above. Industrial procedures for isolation of the sucrose esters were also reported (1,2) but they will not be described since they are not pertinent to this investigation.

Herstein and his workers have reported that the relative proportions of sucrose mono- and diester, obtained from the transesterification reaction, were dependent on the sucrose and catalyst concentrations, and that sucrose diester and sucrose were in equilibrium with sucrose monoester in the reaction solution (4,36). The optimal conditions for the transesterification reaction were also evaluated by orthogonal statistical design (4,36). On the basis of the latter they reached the remarkable

conclusion that, although an equilibrium did exist between sucrose, sucrose monoester and sucrose diester, the sucrose monoester concentration passes through a maximum with increasing sucrose concentration and "with very large excesses of sucrose the yield of monoester decreases, apparently because of increased dilution" (36). Kononenko, moreover, has suggested that the cation of the catalyst formed a chelate complex with sucrose and that this complex was the effective catalyst in the transesterification reaction (4, 36). It was also concluded that the ability of a cation to catalyse the reaction was in some way related to its ionic radius (4, 36). Thus lithium, since it has a smaller ionic radius than potassium, would only give sucrose monoesters since, unlike potassium, it cannot form chelates with two hydroxyl groups at the same time (4,36). Furthermore, the sucrose diester concentration was found to increase with increasing cation-sucrose complex concentration (4,36) at least for those catalysts having a cation with the necessary ionic radius. It should be noted, however, that the conclusions reached by Herstein and coworkers were based entirely on values for the sucrose mono- and diester compositions which were calculated from the saponification equivalents of the sucrose ester mixtures.

An examination of the results of all of the above workers (1,2,3,36) reveals that the reaction times, yields of sucrose ester and product composition vary considerably even for reactions supposedly carried out under identical experimental conditions.

The standard transesterification procedure of Snell (1) has also been the subject of researches by some Japanese workers (38, 39). Komori used acyl pyrrolidines, acyl piperidines, acyl morpholines and alkyl pyrrolidines, as well as DMF, as solvents for the transesterification reaction (38). Formyl morpholine was shown to be a satisfactory solvent for the reaction (38). The rate of transesterification of sucrose and a methyl ester, in the presence of potassium carbonate, was found to increase significantly when the pressure in the system was reduced, presumably due to more efficient removal of methanol from the reaction solution (38). The rate of transesterification also increased, at constant pressure, with an increase in temperature (38). For example, it was shown that a reaction which had gone to completion in two hours at 100° and 35 mm pressure, had only reacted to the extent of 50 percent at 80° and 35 mm pressure (38). Sodium methoxide, potassium carbonate and sodium hydroxide were found to be more efficient catalysts for the reaction than potassium hydroxide or sodium carbonate; the latter was the least

efficient (38). All the rate measurements were determined by following the methyl ester concentration in the reaction solution. Aliquots of the latter were taken at intervals, and the methyl ester extracted with n-hexane. The solvent was subsequently removed and the residue of methyl ester weighed (38).

Mihara and Takaoka have prepared sucrose mono-ricinoleate and mono-12-ketooleate and the corresponding diesters (39). The former were prepared by reacting sucrose and the methyl ester, under the standard conditions (1), in DMF using potassium carbonate as catalyst. The reaction solution was maintained at 80-85° and 80-90 mm pressure for fifteen hours with a stream of carbon dioxide passing through the solution. Unreacted methyl ester was removed by extraction with petroleum ether and the excess sucrose removed by precipitation with acetone (39).

Komori and his coworkers have also prepared a series of sucrose diesters of saturated and unsaturated fatty acids containing fourteen to eighteen carbon atoms (40). In all cases a 1:2 molar ratio of sucrose to methyl ester was used and the reaction was carried out, at 100° and 30 mm pressure, in formyl morpholine using potassium carbonate as a catalyst. The rate of transesterification appeared to be dependent on sucrose concentration. For

example, at the end of two hours the methyl ester had reacted to the extent of 56, 66 and 76 percent when the molar ratio of sucrose to methyl ester was 1:2, 1:1.5 and 1:1, respectively (40). It should be remembered, however, that the method for following the rates of reaction, described above (38), was rather crude.

Some researches have been carried out to determine what side reactions occurred in the transesterification reaction. Billy (3) and Herstein (4,36) have shown that dimethylamine is produced by the action of the inorganic base on DMF. Discoloration of the reaction solution was observed (3,4,36), and this was attributed to the reaction of the inorganic base with small amounts of invert sugar present in the sucrose (36). Hydrolysis of the methyl and sucrose esters occurred when moisture was present in the reaction solution (2,3,4,36). Sucrose did not react with DMF, in the presence of an inorganic base, to form formyl esters of sucrose to a significant extent (3). It has been suggested however that dimethylamine (see above) reacts with the methyl esters of fatty acids to form the corresponding amide (36). The hydrolysis of the methyl esters of fatty acids by sucrose to give the free fatty acids (and water) was suggested since they were isolated from the reaction products (4,36).

The structure of sucrose monolaurate was investigated by Snell and his coworkers (41) in 1956. It was found that one mole of sucrose monolaurate consumed 2.9 moles of periodate with the production of 0.68 moles of formic acid. Tosylation of the sucrose ester in pyridine gave a product containing 3.15 tosyloxy groups per mole of ester. The latter on reaction with sodium iodide in acetone, at 105° in a sealed tube for sixteen hours, gave recoveries of sodium p-toluenesulphonate, for duplicate experiments, in 71.5 and 81 percent yield. These results, however, were based on the assumption that the three primary tosyloxy groups would react with sodium iodide. Sucrose monolaurate was also hydrolysed, at reflux temperature, with 0.5N oxalic acid. Aliquots of the hydrolysis mixture were withdrawn at intervals and extracted with chloroform to isolate the hexose ester, formed by hydrolysis, and unreacted sucrose ester. After removing the chloroform, the residue was deacylated by transesterification with methanol using sodium methoxide as catalyst. After neutralizing the methanolic solutions with acetic acid, the solution was spotted on chromagraphic paper, and the chromatogram was subsequently developed with ethyl acetate-acetic acid-water (3:1:3). The hydrolysis products, namely glucose and fructose, were detected by spraying with aniline phthalate solution.

The concentration of glucose was found to be four times that of fructose as estimated by visual examination of the intensity of the spots. On the basis of this work Snell claimed that at least 80 percent of the lauryl groups, present in the original sucrose monolaurate, was present at the 6-position of glucose (41). It should be noted that this work did not take into account the possibility that ester groups in a sucrose molecule might have very different rates of hydrolysis in acid media or try to give an explanation for the very low recovery of formic acid (theory 1 mole) obtained in the periodate oxidation of the sucrose ester. Billy has shown, however, that only the tosyloxy in the 6-and 6'-positions of sucrose 5.67-tosylate were replaced by iodide ion (3). Reinterpretation of Snell's results on sucrose monolaurate 3.15 tosylate, taking into account this specificity of replacement by iodide ion, revealed that only 37 percent of the lauryl groups in the original sucrose monolaurate could have occupied the 6-and 6'-positions (3). In contrast Billy claimed, on the basis of the replacement of tosyloxy groups by iodine, that 50 percent of the acyl groups were present in the 6- and 6'-positions and the remainder in the 1'-position of sucrose (3).

3. General features of the transesterification reaction

Metathesis is common in inorganic reactions due to the facility with which the ionic bonds are cleaved by highly polar solvents. In organic reactions, on the other hand, a catalyst is often required to assist in the dissociation of covalent bonds. As a result, the organic chemist encounters reactions of a complex and irreversible character more frequently than simple equilibrium (reversible) reactions (42). Ester-ester interchange, halogen interchange in aliphatic halides and possibly alkyl radical interchange in organometallic compounds are known examples of the latter (43). All of these reactions can be carried out, with the aid of a catalyst, in non-polar solvents without undesirable side reactions. Moreover the interchange of radicals takes place in a purely random fashion, and the equilibrium composition, which can be calculated from the law of probability, is independent of the reaction temperature. This random process of interchange has been called the "redistribution reaction" (43) and occurs when the rate constants for the forward and backward reactions are equal (i.e. when the standard free energies of the reactants and products of the reaction are equal). The transesterification reaction, like many other equilibrium reactions (42), differs in that the composition of the product at equilibrium is temperature

dependent and may also depend on the medium in which the reaction is carried out (43). All of the above equilibrium reactions involve the cleavage and formation of two types of bonds, linking two different atom pairs, in each of two distinct molecules. For the trans-esterification reaction these are the O-H bond in the alcohol and the C-O bond in the ester. This type of equilibrium is basically different from that resulting from intramolecular rearrangement in which more than one bond in the molecule is involved in the rearrangement. Examples of the latter are tautomerism, anomerisation, racemisation, cis-trans isomerisation and epimerisation.

A survey of the literature reveals that, although the transesterification reaction is a common tool of the organic chemist (44), only two previous studies of the kinetics and temperature coefficients of this reaction have been made (45,46) and one of these was carried out on the pseudo ester, l-menthyl l-O-benzoyl-benzoate (45). This is in contrast to the many publications on the hydrolysis of esters (47,48,49,50,51, 52,53). There is, however, more information in the literature on the acid catalysed transesterification reaction (46,53,54). In 1950 Taft and his coworkers demonstrated that the transesterification of a series of ortho, meta and para substituted l-menthyl benzoates, with

methanol (using sodium methoxide as the catalyst), was a bimolecular reaction showing first order kinetics in ester and alkoxide ion, respectively. It was also established unequivocally, by the use of an optically active alcohol (l-menthol), that no alkyl oxygen fission occurred in the alcohol (46).

EXPERIMENTAL

All melting points are uncorrected and were determined with a Leitz, hot stage apparatus. The specific rotations were calculated from rotations measured at room temperature, 20-25°, using the D-line of sodium. Infrared spectra were obtained with a Perkin-Elmer, double beam, "Infracord" spectrophotometer. The infrared spectra of compounds were taken in chloroform solution (5% w/v) unless otherwise specified. Determinations of the activity in C^{14}-labelled compounds, using a "Tracerlab (SC-50B) Automatic Flow Counter", as well as microanalysis for carbon, hydrogen, sulphur and iodine, were carried out by Miss E. Busk, Department of Chemistry, University of Ottawa. Gas-liquid partition chromatographic (G.L.P.C.) analyses were obtained with a "Pye Argon Chromatograph" and some of the pertinent characteristics are listed below,

Column size, 4 ft., 5 mm I.D.

Detector, ionization (80 μc radium D)

Carrier gas, Argon

Sample size, 3-6 γ

Resolution, up to 1000 theoretical plates/ft.

Limit of detection, 0.01 γ

The proton magnetic resonance (n.m.r.) spectra were measured, at room temperature, on a Varian V-4302 high resolution

n.m.r. spectrometer, equipped with a field stabilizer, at a fixed frequency of 60 Mc/S. All chemical shifts (τ values) were calculated relative to tetramethyl silane, which was used as an internal standard.

The chemical reagents used in all experiments were reagent grade unless otherwise specified.

Column packings for G.L.P.C. analyses

For all analyses carried out at 200° the chromatographic columns were packed with 10 g of Gelite 545 impregnated with Apiezon M (9:1 w/w) prepared in the manner reported by James and Martin (55). Analyses at 160° were obtained on columns packed with 10 g Gelite 545 impregnated with butanediol succinate polyester (9:1 w/w) (56,57). The chromatographic columns were conditioned, in a stream of argon at 40° above the normal operating temperature for several hours, before they were used.

The retention volumes (V_R) of the methyl esters of the higher fatty acids from C_{14}-C_{18} relative to n-octadecane

A mixture of n-octadecane and the methyl esters of myristic (C_{14}), palmitic (C_{16}) and stearic (C_{18}) acids were separated by G.L.P.C. on the 4 ft. Apiezon M column at 200° using a flowrate of 100 ml argon per minute. The retention volumes of the methyl esters relative to, the

standard compound, n-octadecane were calculated in the usual manner (55) and are given in Table I.

TABLE I

Retention volumes of methyl esters relative to n-octadecane

Compound	$\frac{V_R \text{ methyl ester}}{V_R \text{ n-octadecane}}$
Methyl myristate	0.57
n-octadecane	1.00
Methyl palmitate	1.38
Methyl stearate	3.32

Calibration of the "Pye Argon Chromatograph"

Standard solutions of methyl myristate and n-octadecane were made up in petroleum ether (b.p. 80-100°). Aliquots were pipetted accurately from these standard solutions to give mixtures in the molar ratios listed in Table II. The total volume of each mixture was adjusted so that a 0.1 µl sample when analysed by G.L.P.C. contained a total weight of from 3-6 γ of methyl myristate and n-octadecane. The mixtures were analysed on the 4 ft. Apiezon M column at 200°C with a flowrate of 60 ml argon per minute and the areas under the peaks on the separation curves were measured by means of a planimeter. The molar

ratio of methyl myristate to n-octadecane was compared with the corresponding area ratio obtained from the G.L.P.C. analysis. The numerical values for these ratios are given in Table II and the graph showing the relationship of these ratios is given in Figure II. This method of calibration, using an internal standard, was necessary because it was too difficult to pipette known weights of material in only 0.1 µl of solution.

TABLE II

Molar ratios of methyl myristate to n-octadecane as determined by G.L.P.C.

Calculated	Found (area ratio)
1.735	1.725
1.530	1.530
1.210	1.170
0.621	0.627
0.454	0.446
0.169	0.159
mean deviation =	-0.01 ± 0.02

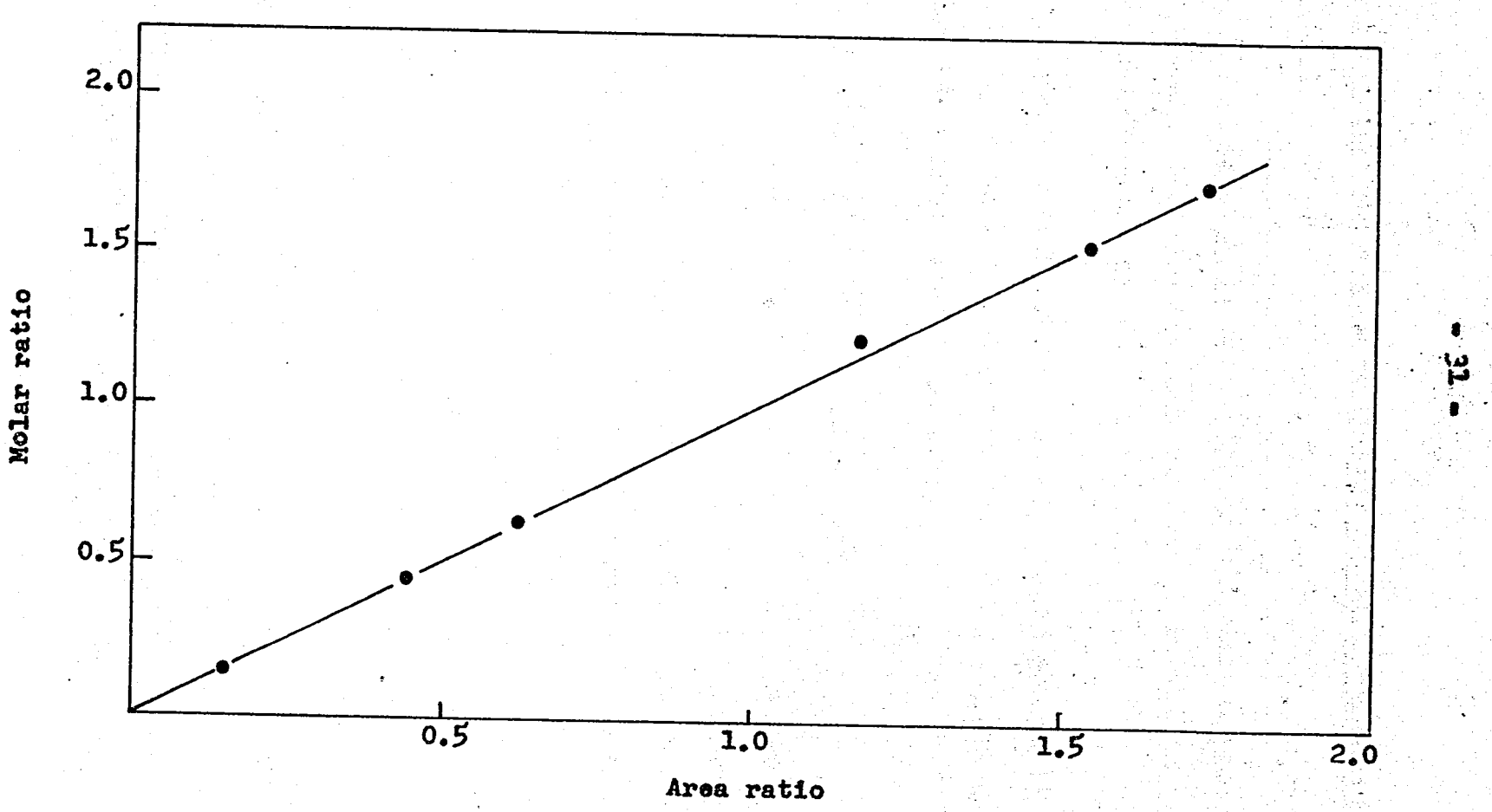


Figure II. The plot of the area ratio (obtained on the "Pye Argon Chromatograph") against the theoretical molar ratio of methyl myristate to n-octadecane.

The purification of materials used in the transesterification reaction

N,N-dimethylformamide was distilled from phosphorous pentoxide. Only the fraction boiling at 153-154° at atmospheric pressure was collected for use.

Sucrose was an ordinary commercial grade, having an $[\alpha]_D$ + 66.50 (c,22: water), and was stored, over phosphorous pentoxide, under reduced pressure.

Methyl esters of fatty acids (C_{14}-C_{18})

Methyl myristate and methyl palmitate had a purity of greater than 99.0 moles % when analysed by G.L.P.C. The former contained methyl laurate and the latter methyl myristate as the impurity. Methyl stearate contained 9.0 moles % of methyl myristate, methyl palmitate and an unidentified ester having a retention volume corresponding to that expected for a C_{17} fatty acid methyl ester. Since there were no free fatty acids present in the methyl esters, the latter were used without further purification.

Potassium carbonate (A.S.C. code 2102; B and A quality) was obtained from Nichols Chemical Company, 1450 City Councillors, Montreal. Before using the potassium carbonate was dried at 180° for 24 hours and was stored, over

phosphorous pentoxide, under reduced pressure.

Methanol was dried by distillation from magnesium. The methanol was used to prepare methanolic solutions of lithium, sodium and potassium methoxides, and also potassium carbonate.

n-Octadecane had a purity greater than 99.5% when analyzed by G.L.P.C.

Petroleum ether (b.p. 80-100°) was redistilled at atmospheric pressure.

Standard apparatus for the transesterification reaction

The apparatus consisted of a four-necked 500 ml round bottom standard-taper flask fitted with a Claisen head to take a mechanical stirrer, operating through a ball and socket joint lubricated with Apiezon M at one arm and a 47 cm. long Vigreaux column (Corning Cat. No. 3525) at the other arm. The other two necks were used for a thermometer and a capillary to enable a stream of nitrogen to be passed through the reaction solution. A glass adapter (Burrel Corp. Cat. No. 264-38) was sealed to the flask to take a silicone rubber seal (Burrel Corp. Cat. No. 261-9). A thermometer was located at the top of the Vigreaux column to record the temperature of any distillate.

A single surface condenser and receiver completed the actual reaction assembly. The latter was connected to a vapour trap which was followed by a manometer and this to a needle valve to control the vacuum. A second vapour trap separated the needle valve and the mechanical pump. The vapour traps were cooled to -70° with a dry ice-acetone mixture. The reaction mixture was maintained at constant temperature by immersing about two-thirds of the reaction flask in a thermostatically controlled oil bath. The apparatus is shown in Plate I.

Standard reaction conditions for the transesterification of sucrose and the methyl esters of fatty acids (C_{14}-C_{18}) in N,N-dimethylformamide (DMF)

The standard apparatus for the transesterification reaction was used. Sucrose (60 mM) was dissolved, under reduced pressure at an oil bath temperature of 89 ± 1°, in 300 ml of anhydrous N,N-dimethylformamide (DMF), with mechanical stirring and while passing a fine stream of nitrogen through the solution. After 30 minutes, the temperature was constant at 80 ± 0.5° and 75 mm pressure. At the end of this period, an aliquot (50 ml) of petroleum ether containing a methyl ester (20 mM) and n-octadecane (12.75 mM) was added to the DMF solution at atmospheric pressure. The petroleum ether was removed

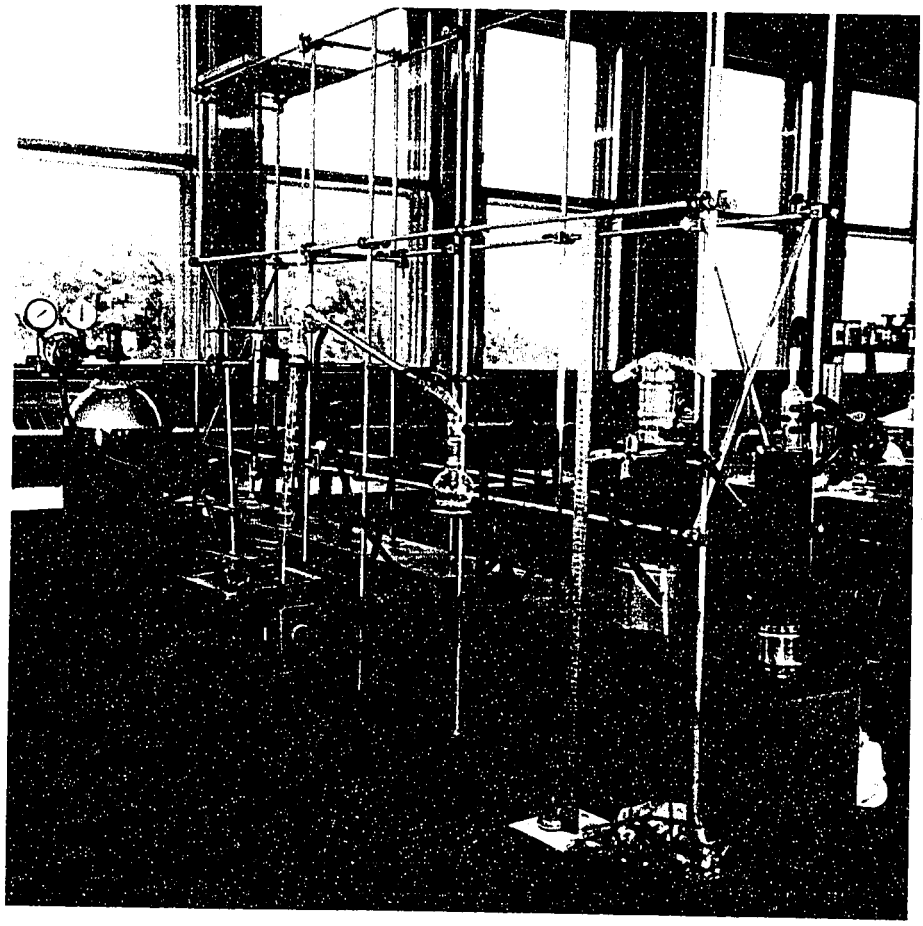


Plate I. The standard transesterification reaction apparatus.

as quickly as possible, under reduced pressure, and the solution left standing until the temperature was constant at 80° (75 mm pressure). This operation took exactly a further 30 minutes. An aliquot (0.6 ml) of the solution was then taken and analysed by G.L.P.C. (see below). The catalyst (4 milliequivalents) in anhydrous methanol (5 ml) was then added to the reaction mixture at 75 mm pressure. This was done by injecting the catalyst solution, with a 5 ml hypodermic syringe, into the reaction mixture through the silicone rubber seal on the reaction vessel. On addition of the catalyst solution the boiler temperature usually dropped to 75-76° and took approximately 10 minutes to return to 80° at 75 mm. pressure. Furthermore, when the catalyst solution was added a white precipitate appeared which took from 2-4 minutes to redissolve completely. During the course of the reaction, the temperature, as measured by the thermometer at the top of the Vigreaux column, remained constant at 29 $\pm$ 1°.

The progress of the reaction was followed at intervals of 30 minutes by following the decrease in concentration of methyl ester by G.L.P.C. using n-octadecane as an internal standard. Furthermore, these analyses were carried out on a 4 ft. Apiezon M column at 200°, under the operating conditions previously described for the calibration of the G.L.P.C. apparatus

(see page 29). Since direct injection of samples of the reaction mixture could have resulted in blockage of the fine capillaries in the injection pipettes, due to possible charring of sucrose and sucrose ester present in the reaction solution, the following procedure was adopted. An aliquot (0.6 ml) of the DMF solution was taken, with a 2 ml hypodermic syringe, fitted with a three inch needle, through the silicone rubber seal without disturbing the vacuum in the apparatus. An aliquot (0.6 ml) of 5% sodium chloride solution was added to the sample and the methyl ester and n-octadecane extracted with an aliquot (0.5 ml) of petroleum ether/ether (3:1) solution. After shaking vigorously, the solution was centrifuged and an aliquot (0.1 µl) of the petroleum ether/ether layer was analysed by gas-liquid partition chromatography. When standard mixtures of methyl myristate and n-octadecane in a 0.2M solution of sucrose in DMF were analysed, by G.L.P.C. in this manner, the results obtained were identical to those reported in Table II.

Since the ionization detector was shown to respond on a molar basis (see Figure I) it was possible to calculate the percentage of methyl ester which had reacted at any given time in the following manner. Suppose A was the initial peak area ratio of methyl ester to n-octadecane and B was the ratio after time t; then the

percentage of methyl ester p which had reacted in time t could be expressed mathematically by,

$$p = 100 (1 - B/A) \qquad (1).$$

The yields of sucrose ester at the end of the reaction time were obtained, in duplicate, on aliquots (25 ml) of the reaction mixture worked up in the following manner. Solid carbon dioxide was added to cool the solution to 20°. At this temperature any residual methyl ester and n-octadecane was extracted quantitatively with three aliquots (50 ml) of petroleum ether. The DMF solution was then taken to dryness, in approximately 10 minutes, at 70° under reduced pressure. The residue was dissolved in n-butanol (25 ml) and washed with two aliquots (25 ml) of 5% sodium chloride solution to remove the potassium carbonate and unreacted sucrose. The combined aqueous phases was extracted with a further aliquot of n-butanol (15 ml). The n-butanol solutions were then combined. Any sodium chloride in the n-butanol solution was precipitated by the addition of chloroform (40 ml) and the chloroform/n-butanol solution was dried with anhydrous sodium sulphate (10 g). After standing overnight the solution was filtered; the material on the filter being washed thoroughly with a further aliquot of chloroform (40 ml). At this stage the

chloroform/n-butanol solution (120 ml) was usually clear with no precipitate present. If a precipitate was present the solution was refiltered through acid washed Celite 545 (5 g; 80-100 mesh). The solution was taken to dryness under reduced pressure, and the last traces of n-butanol and DMF removed by azeotropic distillation with ethanol. The white residue of sucrose ester was dried, over phosphorous pentoxide, under reduced pressure, and weighed.

The total yield of sucrose ester obtained from the reaction of sucrose (60 mM) and methyl ester (20 mM) was calculated as follows. If $\underline{w}$ was the weight of sucrose ester in a 25 ml aliquot of the DMF solution; $\underline{v}$ the total volume of samples taken for G.L.P.C. analyses and $\underline{V}$ the total volume of DMF solution left after all samples were taken for analyses; then the total weight $\underline{W}$ of sucrose ester can be expressed by the equation

$$\underline{W} = \underline{w}\,(50 + \underline{v} + \underline{V})/25 \qquad (2).$$

Furthermore, if $\underline{W_1}$ was the theoretical yield of sucrose monoester (20 mM), then $\underline{P}$, the percentage yield based on the complete conversion of methyl ester (20 mM) to sucrose monoester, was given by,

$$\underline{P} = 100\underline{W}/\underline{W_1} \qquad (3).$$

The percentage yield ($\underline{P}_1$) of sucrose ester based on the amount of methyl myristate which reacted was given by

$$\underline{P}_1 = 100\underline{P}/\underline{P}_2 \qquad (4)$$

where $\underline{P}_2$ was the percentage of methyl ester which had reacted.

Routine tests for the presence of impurities in the sucrose ester

1) Sucrose ester (50 mg) was dissolved in 1 ml chloroform at room temperature. In the absence of sucrose and sodium chloride a clear solution, with no insoluble residue, was obtained.
2) A 0.1 µl aliquot of the above chloroform solution was analyzed by G.L.P.C. for methyl ester and n-octadecane.
3) An infrared spectrum of the above chloroform solution was taken. The presence of DMF or the anion of a fatty acid could be detected since they have characteristic absorptions, for the c = o stretching vibration, at approximately 1670 cm^{-1} and 1560 cm^{-1}, respectively.
4) An aqueous solution of the sucrose ester was tested for chloride ion with silver nitrate.

The transesterification of sucrose with methyl myristate in DMF using solid potassium carbonate as the catalyst

The apparatus and methods for following the transesterification reaction were the same as that described previously. However, since no provision was made in the original apparatus for adding a solid catalyst when the apparatus was under reduced pressure, the following procedure was adopted. After the customary conditioning period of one hour, the apparatus was brought to atmospheric pressure and the solid catalyst added, to the reaction solution, through the neck in the reaction flask usually carrying a thermometer. The latter was then replaced immediately and the reaction solution brought as quickly as possible to the standard operating condition of 80° at 75 mm pressure. Furthermore the usual concentrations of sucrose (60 mM), methyl myristate (20 mM), n-octadecane (12.75 mM), potassium carbonate (4 milliequivalents) and 300 ml of anhydrous DMF were used.

The standard reaction conditions were modified, however, to determine the effect of passing nitrogen through the reaction solution, mechanical stirring and the physical state of the catalyst, on the rate of the reaction and yield of sucrose myristate. It was considered

that a study of these variables would establish whether the transesterification procedure reported by Snell (1), using solid potassium carbonate as catalyst could be carried out in a reproducible manner. The four experimental conditions, which were investigated are given below.

a) The reaction was carried out without mechanical stirring or nitrogen passing through the solution.

b) The reaction conditions were the same as for (a) except that nitrogen was passed through the solution.

c) The reaction solution was stirred mechanically and had nitrogen passing through it.

d) The reaction conditions were identical to (c) except that the potassium carbonate was impregnated on 1 g of Celite. This was done by mixing 1 ml of water containing 4 milliequivalents of potassium carbonate with 1 g of acid washed Celite 535. After drying in an oven, at 180° for 48 hours, it was stored under reduced pressure over phosphorous pentoxide.

Typical results for the above experimental conditions are given in Tables III and IV. The former shows the percentage of methyl myristate which had reacted at intervals of one hour, and the latter gives the yields of sucrose myristate. One experiment from each of the categories is shown graphically in Figure III.

TABLE III

The rate of reaction using solid potassium carbonate as catalyst at 80°

Percentage of methyl myristate reacted

	Experimental condition (1)					
Time		(a)		(b)	(c)	(d)
(hr)	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6
1	7.0	3.5	23.0	48.0	32.0	53.0
2	34.0	19.0	42.4	70.0	60.0	82.1
3	53.5	35.5	53.1	83.3	81.4	91.0
4	59.0	38.0	63.8	88.2	91.9	95.5 (2)
5	71.0	42.0	75.8	93.5	-	
6	82.5(2)	53.5	82.0	95.8	-	
7		58.0(2)	86.4		100.0(2,3)	
11			-	100.0(2,3)		
24			100.0(2,3)			

1. These were the experimental conditions described on page 42.
2. The reaction mixture was analysed at this time for sucrose myristate.
3. Values reported as 100% showed the presence of trace amounts of methyl myristate.

TABLE IV

Percentage yield of sucrose myristate using solid potassium carbonate as catalyst(1)

Experiment number	1	2	3	4	5	6
Yield based on complete conversion of methyl myristate (20 mM) to sucrose monomyristate	63.0	41.2	70.5	80.5	87.8	79.0
Yield based on the amount of methyl myristate reacted	76.4	71.1	70.5	80.5	87.8	82.8

1. The yields of sucrose myristate correspond to the reaction times shown in Table III.

It should be noted however that the solid potassium carbonate, used as catalyst, did not dissolve to any appreciable extent for any of the above reactions.

The effect of water on the transesterification of sucrose with methyl myristate using solid potassium carbonate as catalyst

The reaction conditions were the same as those described previously. The reaction solution was stirred mechanically and a stream of dry nitrogen was passed through the reaction mixture.

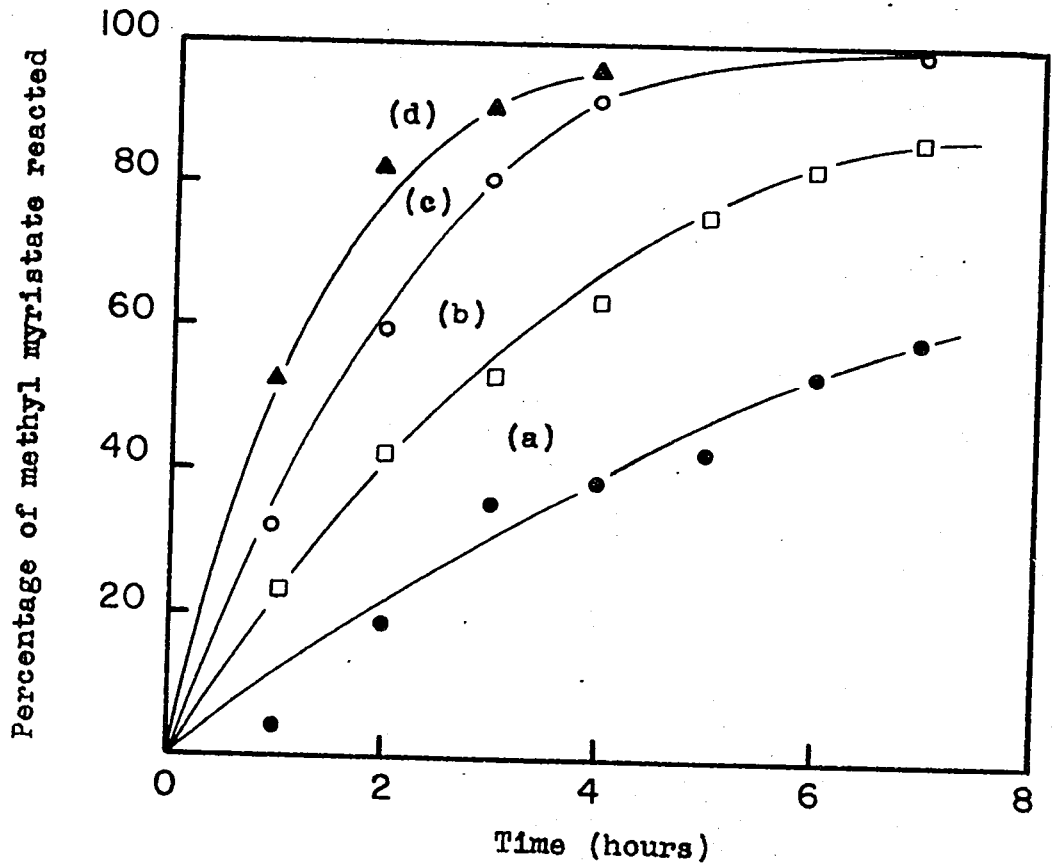


Figure III. Rates of transesterification at 80° using four milliequivalents of solid potassium carbonate as the catalyst. The reaction solution was stirred mechanically in (c) and (d), and had a stream of nitrogen passing through it in (b), (c), and (d). The catalyst in (d) was impregnated on Celite.

The initial concentration of water in the reaction solution was 0.01, 0.05, 0.25 and 1.25% in experiments 7, 8, 9 and 10, respectively. This corresponded to the addition of 0.42, 2.10, 10.5 and 52.5 mM of water per milliequivalent of solid potassium carbonate. The water was added to the reaction solution after the usual one hour conditioning period, and at the same time as the catalyst. It was observed that the solid potassium carbonate did not dissolve completely in any of the experiments.

The results are given in Tables V and VI, and the effect of water on the rate of reaction is shown graphically in Figure IV.

Miscellaneous transesterification reactions using solid potassium carbonate or sodium hydride as the catalyst

Two experiments were carried out to determine whether or not the transesterification reaction was susceptible to homogeneous catalysis. Solutions of potassium carbonate in DMF, or DMF containing sucrose, were therefore prepared (see below) prior to addition of the other reagents. The apparatus and methods of following the reaction were the same as those previously described.

TABLE V

The effect of water on the rate of reaction at 80°

Percentage of methyl myristate reacted

Time (hour)	Experiment number (2)			
	7	8	9	10
1	22.9	16.6	12.0	20.0
2	38.9	36.8	18.5	21.4
3	54.5	49.4	27.6	24.6
4	63.4	57.9	29.2	25.8
5	69.0	64.0	35.2	27.2
6	76.6	71.6	36.2	28.9
7	-	-	37.4	32.8
8	-	-	-	-
9	87.6(1)	88.3	46.7(1)	34.6(1)
10		-		
11		92.9(1)		

1. Aliquots of the reaction mixture were taken to determine the sucrose myristate content.
2. Experiments 7, 8, 9 and 10 contained 0.01, 0.25, 0.5 and 1.25% of water, respectively.

TABLE VI

The effect of water on the yield of sucrose myristate

Experiment number	Percentage yield of sucrose myristate[1]			
	7	8	9	10
Yield based on complete conversion of methyl myristate (20 mM) to sucrose monomyristate	82.2	81.5	31.0	32.3
Yield based on amount of methyl myristate which reacted	93.8	87.7	66.5	93.5

1. The yields of sucrose myristate correspond to the reaction times shown in Table V.

e) The potassium carbonate (8 milliequivalents) and anhydrous DMF (600 ml) were heated together at reflux temperature, at atmospheric pressure, for 18 hours. After the hot solution was filtered an aliquot of 300 ml was used for the transesterification reaction. Two aliquots (25 ml) of the solution of potassium carbonate in DMF were taken to dryness under reduced pressure and the last traces of DMF were removed by azeotropic distillation with ethanol. The residue was dissolved in distilled water (5 ml) and titrated with 0.01N HCl using methyl orange as an

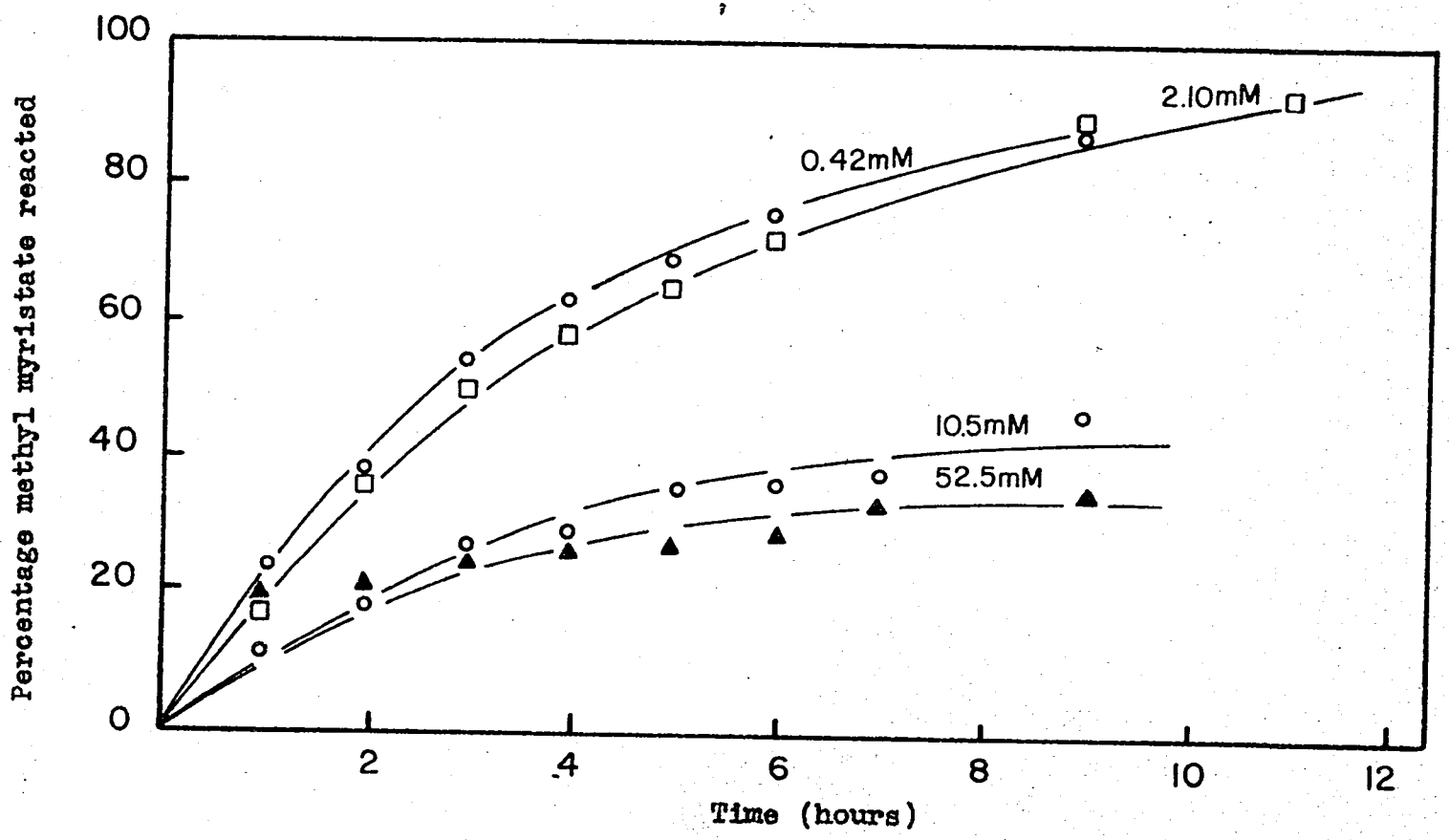


Figure IV. The effect of adding 0.42, 2.10, 10.5 and 52.5 mM of water on the rate of transesterification at 80°, using four milliequivalents of solid potassium carbonate as the catalyst. The reaction solution was stirred mechanically and had nitrogen passing through it.

e) Cont'd.

indicator. The solution was found to be only 0.0014N in potassium ion.

f) The potassium carbonate (4 milliequivalents), DMF (300 ml) and sucrose (60 mM) were heated, at atmospheric pressure, in an oil bath at 80° for 6 hours. After standing overnight, any solids were removed by filtration and the resulting solution was used for the transesterification reaction..

Another two experiments were carried out to see whether the rate of transesterification could be accelerated by carrying out the reaction in a rotary evaporator. Both solid potassium carbonate and sodium hydride were used as catalyst. The latter was used to determine whether sucrate ion would catalyse the reaction.

g) Sucrose (60 mM) was dissolved in anhydrous DMF (300 ml) at 80° in an oil bath, and methyl myristate (20 mM) was added together with solid potassium carbonate (4 milliequivalents). The standard-taper, round-bottom flask (1 l) containing the reaction mixture was then connected to a rotary evaporator fitted with a manostat. The reaction flask was heated with a boiling water bath, and maintained at a vacuum of 190 mm pressure by means of a water pump. Considerable foaming occurred at lower pressures. After six hours

g) Cont'd.

the reaction was stopped and the sucrose myristate isolated from two aliquots (25 ml) of the reaction mixture as described previously (see page 38. An aliquot (2.5 ml) of the reaction mixture was also taken for the estimation of methyl myristate.

h) Another experiment was carried out under the same experimental conditions as (g) except that sodium hydride (4 milliequivalents) was used as the catalyst.

In all of the above experiments great care was taken to carry out the reactions under anhydrous conditions. The results of these experiments are given in Tables VII and VIII.

<u>The transesterification reaction of sucrose with methyl myristate in DMF using dilute methanolic solutions of potassium carbonate, potassium methoxide and potassium hydroxide as the catalyst</u>

All of the reactions reported in Tables IX and X were carried out in homogeneous solution and under identical conditions of temperature, pressure, mechanical stirring and with nitrogen bubbling through the reaction solution as described previously (see page 34). The reactions were carried out under anhydrous conditions and only differed in the catalyst used to promote

TABLE VII

The rate of reaction using solid sodium hydride and potassium carbonate as catalyst at 80°

Percentage of methyl myristate reacted

Time (hour)	Experimental conditions (1) (e) Exp. 11	(f) Exp. 12	(g) Exp. 13	(h) Exp. 14
1	9.9	37.7	-	-
2	9.9	48.8	-	-
3	9.9	-	-	-
4	9.9(2)	51.9	-	
5	-	55.6	-	
6		56.0(2)	66.0(2)	52.0(2)

1. These are the experimental conditions described on pages 50 and 51.
2. The reaction was stopped and analysed for sucrose myristate.

TABLE VIII

Percentage yield of sucrose myristate using solid sodium hydride and potassium carbonate as catalyst[1]

Experiment number	11	12	13	14
Yield based on complete conversion of methyl myristate to sucrose monomyristate	9.5	48.4	52.5	40.8
Yield based on the amount of methyl myristate reacted	96.2	86.3	79.6	78.5

1. These yields correspond to the reaction times given in Table VII.

transesterification of sucrose and methyl myristate. Methanolic potassium carbonate was used in experiments 15 and 16; methanolic potassium methoxide was used in experiments 17 and 18; and methanolic potassium hydroxide was used in experiment 19. The catalyst (4 milliequivalents) was added to the reaction mixture as a solution in anhydrous methanol (5 ml).

In all of the above experiments the clear reaction solution became milky, due to the presence of a fine precipitate, as soon as the methanolic solution of the

catalyst was added. This precipitate, however, redissolved in the DMF solution within 5 minutes.

The methyl myristate concentration was followed at intervals of 30 minutes and the results are given in Table IX. Table X shows the isolated yields of sucrose myristate at the end of each reaction. It is of interest to note that the infrared spectra of the sucrose myristates isolated in these experiments were superimposable.

TABLE IX

The rate of reaction using dilute methanolic solutions of the catalyst at 80°

Percentage of methyl myristate reacted

Time (minutes)	Experiment number (1)				
	15	16	17	18	19
30	37.0	41.7	31.9	35.8	31.4
60	61.2	64.8	55.1	56.2	59.2
90	79.0	78.0	77.2	76.0	74.2
120	85.3	85.7	87.8	84.3	82.5
150	92.9	92.0	92.6	91.4	85.8

1. Experiments 15 (and 16), 17 (and 18) and 19 used methanolic solutions of potassium carbonate, potassium methoxide and potassium hydroxide, respectively.

TABLE X

Percentage yield of sucrose myristate using dilute methanolic solutions of the catalyst

Experiment number	15	16	17	18	19
Yield based on complete conversion of methyl myristate (20 mM) to sucrose monomyristate after 150 minutes	83.8	84.0	84.5	82.1	74.4
Yield based on the amount of methyl myristate which reacted after 150 minutes	90.3	91.3	91.2	89.9	86.7

The effect of using saturated aqueous and methanolic solutions of the catalyst on the rate of transesterification

The following experiments were designed to establish the most satisfactory form of adding potassium ion (4 milliequivalents) to the reaction solution. The standard reaction conditions and methods for following decrease in concentration of methyl ester were used. Sucrose (60 mM) was reacted with methyl myristate (20 mM) in anhydrous DMF using aqueous solutions of potassium carbonate and potassium hydroxide as well as methanolic solutions of potassium hydroxide and potassium methoxide as the catalyst. In experiments 21 and 22 the catalyst

was added in the form of a saturated aqueous potassium hydroxide solution (0.260 ml contained 4 milliequivalents) whereas in experiment 23 it was added as a saturated aqueous solution of potassium carbonate (0.329 ml contained 4 milliequivalents). Experiments 20 and 24 used saturated methanolic solutions of potassium hydroxide (0.560 ml contained 4 milliequivalents) and potassium methoxide (0.68 ml contained 4 milliequivalents).

A fine precipitate appeared on addition of the catalyst giving the reaction solution a milky appearance in all experiments (20 and 24) in which a methanolic solution was used. This precipitate redissolved within a period of 5 minutes. However, only a small amount of precipitate appeared on adding aqueous potassium hydroxide (experiments 21 and 22) to the reaction solution and this had redissolved by the end of the reaction period. No precipitate appeared when aqueous potassium carbonate was used (experiment 23).

The rate of reaction and yields of sucrose myristate are given in Tables XI and XII.

TABLE XI

The effect of using saturated solutions of the catalyst on the rate of reaction at 80°

Percentage of methyl myristate reacted

Time (minutes)	Experiment number 20(1)	21	(2) 22	23(3)	24(4)
30	39.8	34.5	28.4	27.7	58.5
60	58.3	53.8	48.3	50.4	78.9
90	67.2	63.5	61.2	66.7	87.1
120	72.8	72.9	70.8	73.6	94.0
150	76.9	79.6	76.7	79.1	96.9

1) and 4) Saturated methanolic solutions of potassium hydroxide and potassium methoxide were used as catalyst, respectively.

2) and 3) Saturated aqueous solutions of potassium hydroxide and potassium carbonate were used as catalyst, respectively.

TABLE XII

The effect of using saturated solutions of the catalyst on the yield of sucrose myristate

Percentage yield of sucrose myristate

Experiment number	20	21	22	23	24
Yield based on complete conversion of methyl myristate to sucrose mono-myristate after 150 minutes	69.5	72.5	72.7	71.2	81.7
Yield based on amount of methyl myristate reacted after 150 minutes	90.4	91.0	94.8	90.0	84.5

The effect of sucrose concentration on the rate of the transesterification reaction

The standard reaction conditions and methods of analysis were used, except that the experiments were carried out using 60 mM, 40 mM and 20 mM of sucrose, respectively. Sucrose was dissolved in anhydrous DMF (300 ml) and reacted with methyl myristate (20 mM), at 80° and 75 mm pressure, using a saturated methanolic solution of potassium methoxide (0.68 ml containing 4 milliequivalents) as catalyst.

In all experiments a fine white precipitate appeared immediately the catalyst was added to the reaction solution. When 60 mM of sucrose was used the catalyst redissolved rapidly and the reaction solution was homogeneous after 5 minutes. This was true also of the experiment in which 40 mM of sucrose was used although in this case a small amount of precipitate was present at the end of the reaction. Substantial amounts of precipitate formed however in the case of the experiment using 20 mM of sucrose and only dissolved slowly as the reaction proceeded. In the latter case the residual precipitate (185.3 mg) was collected on a filter and washed with acetone and chloroform and then dried, in vacuo at 78°, under reduced pressure. Two 10 mg samples, in 5 ml of distilled water, were titrated with 0.01N HCl using phenophthalein, as an indicator. The neutralization equivalent of the precipitate was found to be 769 suggesting that it was in fact a mixture of sucrose and potassium sucrate. The presence of sucrose was confirmed by taking the infrared spectrum, as a Nujol mull, of the precipitate and comparing it with the spectrumof an authentic sample of sucrose.

The results of these experiments are given in Tables XIII and XIV.

TABLE XIII

The effect of sucrose concentration on the rate of reaction at 80°

Percentage of methyl myristate reacted

Time (minutes)	60 mM Sucrose Exp. 24	40 mM Sucrose Exp. 25	20 mM (1) Sucrose Exp. 26
30	58.5	55.0	38.5
60	78.9	75.1	57.6
90	87.1	83.5	76.8
120	94.0	91.4	83.9
150	96.9	95.5	84.8

1. Substantial amounts of catalyst precipitated from solution and did not redissolve during the course of reaction.

TABLE XIV

The effect of sucrose concentration on the yield of sucrose myristate

Percentage yield of sucrose myristate

Experiment number	24	25	26(1)
Yield based on complete conversion of methyl myristate to sucrose monomyristate after 150 minutes	81.7	84.5	68.7
Yield based on amount of methyl myristate reacted after 150 minutes	84.5	89.0	76.5

1. Substantial amounts of catalyst precipitated from solution and did not redissolve during the course of the reaction.

The effect of the cation of the catalyst on the rate of reaction

Experiments 15, 27, 28 and 18 were carried out with a view to establishing whether the cation of the catalyst influences the reaction rate. The standard apparatus, reaction conditions and methods of analysis were used except that potassium carbonate, lithium methoxide, sodium methoxide and potassium methoxide

(4 milliequivalents) in methanol (5 ml) were used as the catalyst, respectively. Sucrose (60 mM) was reacted with methyl myristate (20 mM) in anhydrous DMF (300 ml), at 80° and 75 mm pressure, using the above catalysts, and n-octadecane (12.75 mM) as an internal standard.

All of the methanolic solutions of the catalysts, except lithium methoxide, gave an initial precipitate when added to the reaction solution. These precipitates all redissolved rapidly (within 5 minutes).

The results of these experiments are shown in Table XV.

TABLE XV

The effect of the cation of the catalyst on the rate of reaction at 80°

Percentage of methyl myristate reacted

Time (minutes)	K_2CO_3, MeOH Exp. 15	LiOMe Exp. 27	NaOMe Exp. 28	KOMe Exp. 18
30	37.0	37.9	37.9	35.8
60	61.2	58.0	62.2	56.2
90	79.0	74.8	77.8	76.0
120	85.3	85.2	89.1	84.3
150	92.9	90.7	94.9	91.4

The effect of gaseous carbon dioxide passing through the reaction solution

The reaction conditions were the same as for experiment 15 (see Table IX) except that carbon dioxide was passed through the solution instead of nitrogen during the normal reaction time of 2-1/2 hours. Aliquots of the reaction solution were then taken to determine the sucrose myristate content. The carbon dioxide was then replaced with nitrogen and the reaction continued for a further 2 hours. At the end of this period the sucrose myristate content was determined again. An initial precipitate formed when the potassium carbonate (4 milliequivalents) in methanol (5 ml) was added to the reaction mixture and then redissolved (5 minutes) to give a homogeneous solution.

The results for experiments 15 and 29 are shown in Tables XVI and XVII and is represented graphically in Figure V.

TABLE XVI

Effect of carbon dioxide on the rate of reaction

Percentage of methyl myristate reacted

Time (minutes)	Experiment number Exp. 29	Exp. 15
	CO_2	N_2
30	6.8	37.0
60	11.0	61.2
90	13.3	79.0
120	21.1	85.3
150	21.7 (1)	92.9 (1)
	N_2	
180	62.8	
210	82.6	
240	90.3	
280	95.6 (1)	

1. Aliquots of the reaction mixture were taken to determine the sucrose myristate content.

TABLE XVII

The effect of carbon dioxide on the percentage yield of sucrose myristate(1)

Experiment number	29		15
Reaction Time (minutes)	150	280	150
Yield based on complete conversion of methyl myristate to sucrose monomyristate	19.3	84.7	83.8
Yield based on amount of methyl myristate which reacted	89.0	88.6	90.3

1. The yields of sucrose myristate correspond to the reaction conditions and times shown in Table XVI.

The effect of fatty acid chain length on the rate of reaction

Experiments 30, 31 and 32 were carried out using 20 mM of methyl myristate, methyl palmitate and methyl stearate, respectively. In all other respects the standard transesterification reaction conditions were used. Potassium methoxide (4 milliequivalents) in anhydrous methanol (5 ml) was used as catalyst. An initial precipitate formed in all of the experiments when the methanolic solution was added to the DMF/sucrose solution,

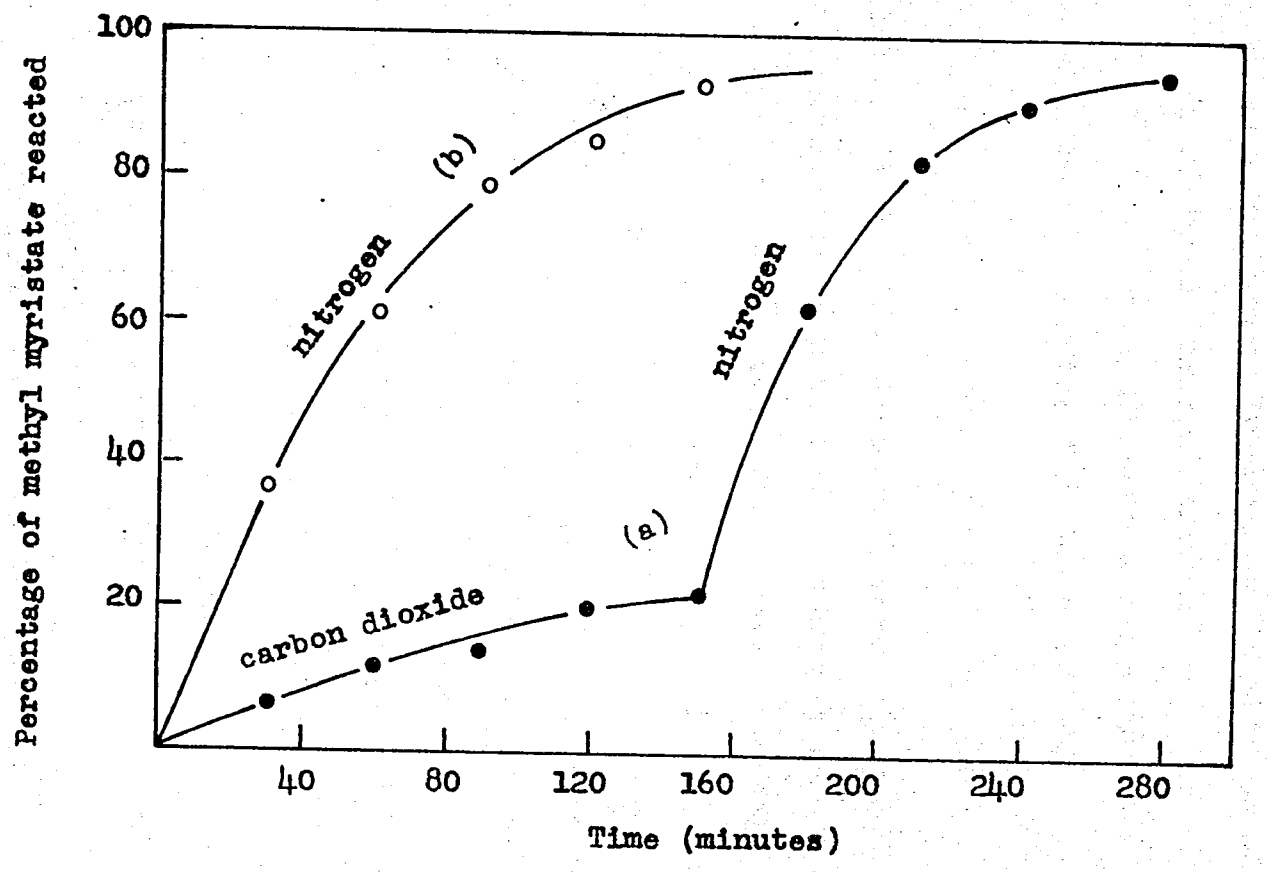


Figure V. Rates of the standard transesterification reaction at 80° using 4 milliequivalents of potassium carbonate, under conditions for homogeneous catalysis. In (a) a stream of carbon dioxide and nitrogen, respectively, was passed successively through the solution, whereas in (b) only nitrogen was used.

and then redissolved rapidly to give a homogeneous solution. The rate of the reaction was determined by G.L.P.C., in the usual manner, by following the methyl ester concentration at intervals of 30 minutes using n-octadecane as an internal standard.

The first order rate constants for the above reaction were calculated from the equation,

$$k_r = 2.303/\underline{t} \log \underline{a}/(\underline{a} - \underline{x}) \qquad (5)$$

where k_r is the first order rate constant; $\underline{a}$ is the initial concentration of methyl ester and $\underline{x}$ is the concentration after time $\underline{t}$ (58). However since $\underline{a}/(\underline{a} - \underline{x})$ is a ratio of concentrations its value will be independent of the units used to express the concentrations provided the same units are used for $\underline{a}$ and $\underline{x}$. The first order rate constant can therefore be calculated by taking the ratio of the percentages of methyl ester in the reaction solution at zero time and time $\underline{t}$. It follows that,

$$\underline{a}/(\underline{a} - \underline{x}) = 100/(100 - \underline{p}) \qquad (6)$$

where $\underline{p}$ is the percentage of methyl ester which has reacted in time $\underline{t}$. The latter can be calculated from equation 1 (see page 38).

The results are given in Table XVIII.

TABLE XVIII

The effect of the fatty acid chain length on the rate of reaction at 80°

Percentage methyl myristate reacted

Time (minutes)	Percentage of methyl ester reacted[1]			First order rate constant (sec^{-1}) x 10^4		
	M.M.	M.P.	M.S.	M.M.	M.P.	M.S.
30	43.3	43.8	42.4	3.15	3.20	3.07
60	67.5	68.6	63.1	3.12	3.22	2.77
90	84.9	81.7	79.6	3.50	3.15	2.94
120	91.6	89.8	88.7	3.44	3.49	3.04
150	95.5	92.4	-	3.44	2.80	-
Average first order rate constant (sec^{-1}) x 10^4				3.33	3.17	2.96

1. Methyl myristate (M.M.), methyl palmitate (M.P.) and methyl stearate (M.S.) were used in experiments 30, 31 and 32, respectively.

The effect of the catalyst concentration on the rate of reaction

Experiments 33, 34, 35, 30 and 36 were carried out under the standard reaction conditions except that 0.5, 1.0, 2.0, 4.0 and 8.0 milliequivalents of potassium methoxide in anhydrous methanol (5 ml) were used as

catalyst, respectively. The precipitate which appeared on adding the methanolic solution of the catalyst, redissolved rapidly (< 5 minutes) in all experiments except in the case when 8.0 milliequivalents of potassium methoxide was used. In the latter case a large amount of precipitate was present during the entire reaction period.

The results are tabulated in Table XIX and is illustrated graphically in Figure VI.

The effect of temperature on the rate of reaction

In experiments 37 and 38 the transesterification of sucrose (60 mM) and methyl myristate (20 mM) was carried out, at 97° ± 0.5° and 140 mm pressure, using potassium methoxide (2 and 4 milliequivalents, respectively) in anhydrous methanol (5 ml) as catalyst. In all other respects the standard reaction conditions and methods of analysis were used. It was observed that the initial precipitate of catalyst at the 4 milliequivalent level took much longer to dissolve at 97° (45 minutes) than at 80° (3 minutes). The decrease in concentration of methyl myristate was followed by G.L.P.C. at intervals of 15 minutes, using n-octadecane as the internal standard.

TABLE XIX

The effect of catalyst concentration on the rate of reaction at 80°

Time (minutes)	Percentage of methyl myristate reacted (1)					First order rate constant (sec^{-1}) x 10^4 (1)				
	0.5 m.e.	1.0 m.e.	2.0 m.e.	4.0 m.e.	8.0 m.e.	0.5 m.e.	1.0 m.e.	2.0 m.e.	4.0 m.e.	8.0 m.e.
30	13.2	20.0	31.7	43.3	44.8	0.79	1.24	2.12	3.15	3.30
60	22.3	37.2	54.5	67.5	72.4	0.70	1.29	2.19	3.12	3.58
90	35.4	57.4	70.0	84.9	85.6	0.79	1.58	2.23	3.50	3.59
120	43.9	67.1	82.6	91.6	93.9	0.80	1.54	2.43	3.44	3.89
150	49.8	74.2	89.0	95.5	97.3	0.77	1.51	2.45	3.44	4.01
Average first order rate constants (sec^{-1}) x 10^4						0.75	1.43	2.36	3.33	3.67

1. Experiments 33, 34, 35, 30 and 36 used 0.5, 1.0, 2.0, 4.0 and 8.0 milliequivalents (m.e.) of potassium methoxide as catalyst, respectively.

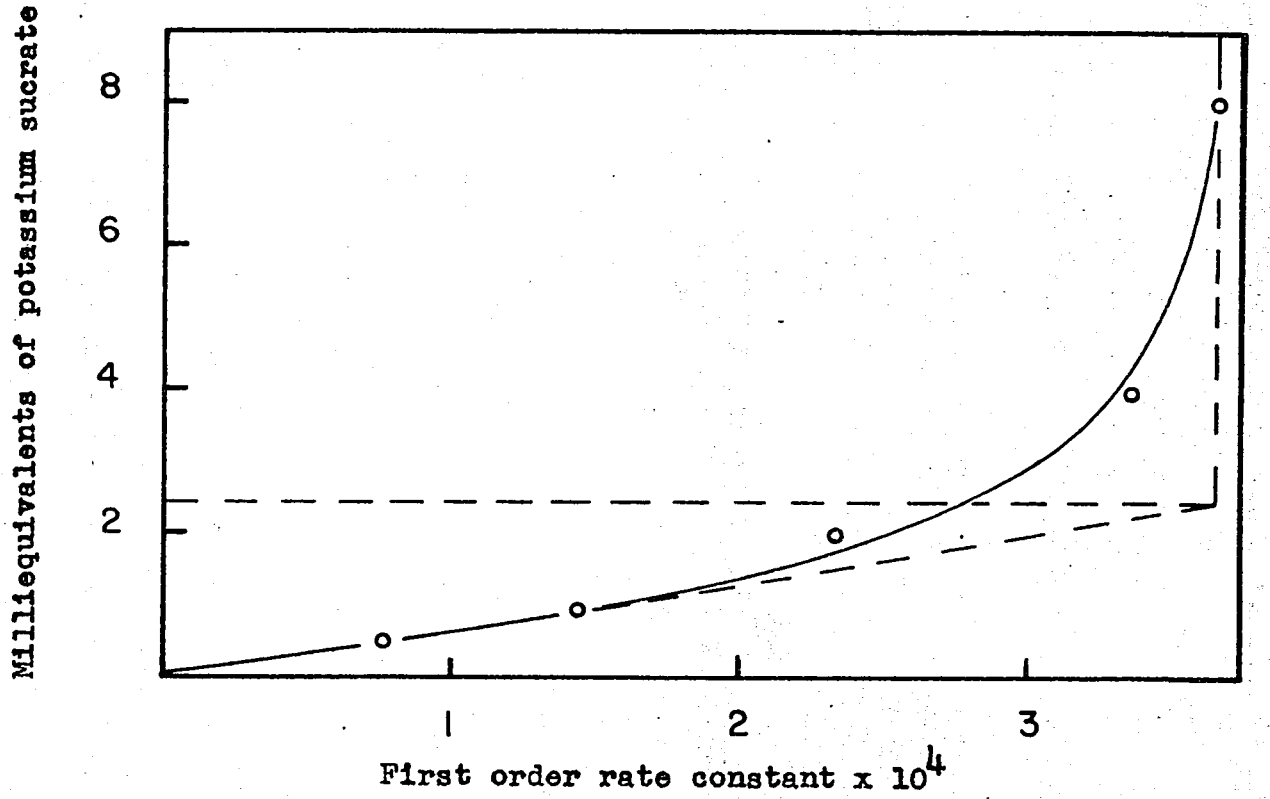


Figure VI. The plot of the pseudo first order rate constants for the standard transesterification reaction at 80° against the potassium sucrate concentration in 320 ml of reaction solution.

The first order rate constants are given in Table XX. The effect of temperature on the rate of the reaction is shown in Figure VII and VIII.

TABLE XX

The rate of transesterification at 97° (1)

Time (minutes)	Percentage of methyl myristate reacted 2 m.e. Exp. 37	4 m.e. Exp. 38	First order rate constant (sec^{-1}) x 10^4 2 m.e. Exp. 37	4 m.e.(2) Exp. 38
15	31.6	38.8	4.22	5.47
30	54.0	61.3	4.36	5.27
45	68.8	77.6	4.31	5.54
60	82.1	86.8	4.78	5.63
75	88.5	92.3	4.80	5.69
Average first order rate constant x 10^4 (sec^{-1})			4.51	5.52

1. The first order rate constants for the corresponding reactions carried out at 80° were 2.36 x 10^{-4} sec^{-1} and 3.33 x 10^{-4} sec^{-1} respectively (see experiments 35 and 30, Table XIX).
2. Experiments 37 and 38 used 2 and 4 milliequivalents (m.e.) of potassium methoxide as catalyst, respectively.

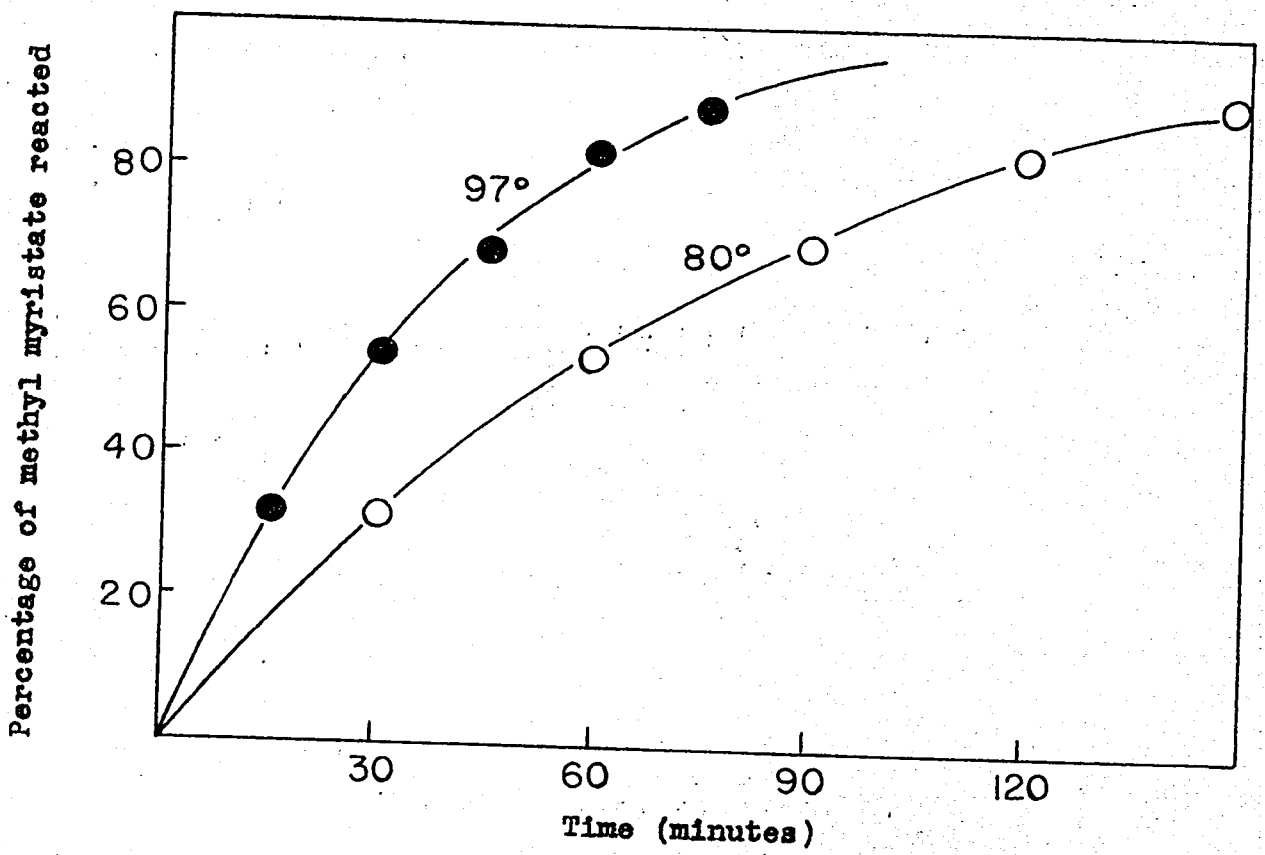


Figure VII. The effect of temperature on the rate of the standard transesterification reaction when two milliequivalents of potassium sucrate was used as catalyst.

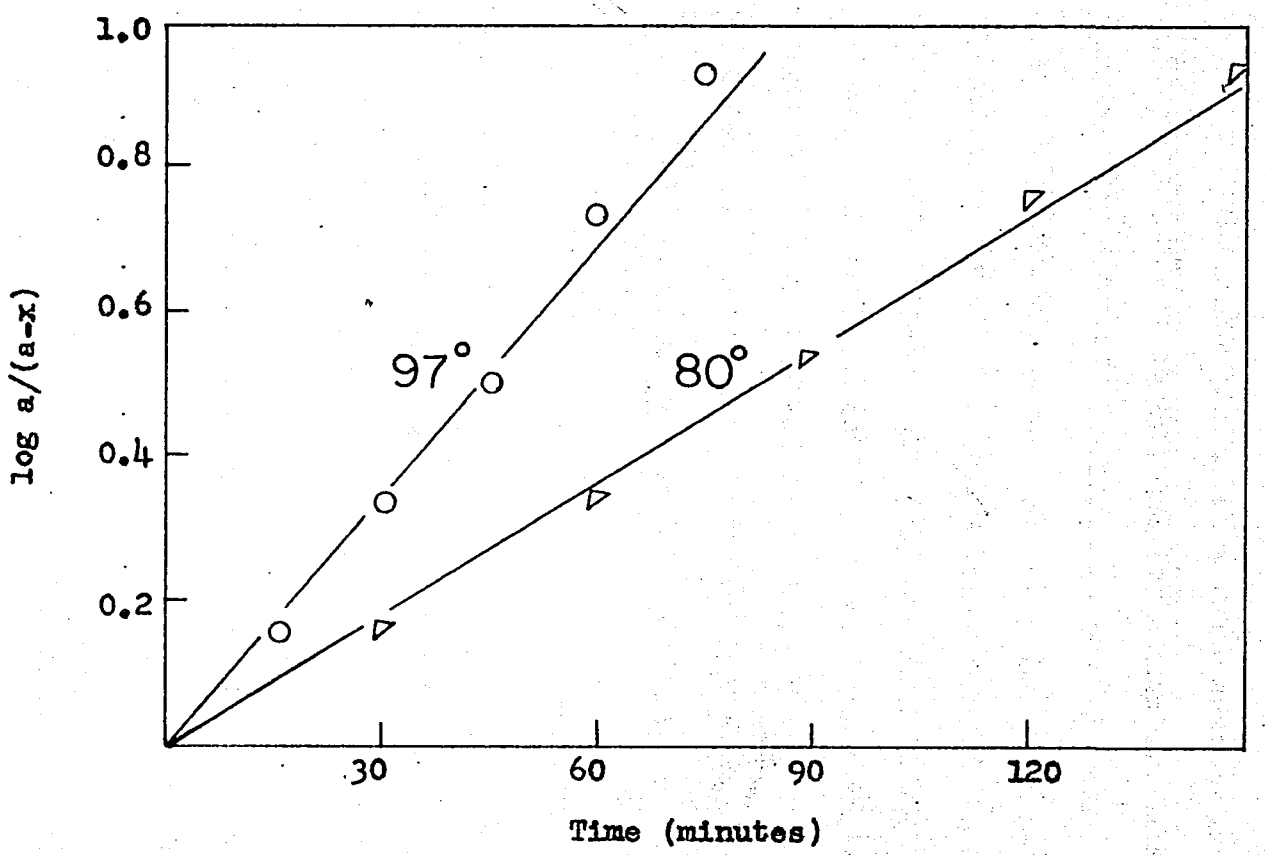


Figure VIII. The plot of log a/(a-x) against time for the transesterification reaction, at the two milliequivalent level of potassium sucrate, showing the pseudo first order kinetics of methyl myristate in the reaction and the effect of temperature.

The activation energy for the transesterification reaction

The first order rate constants were 2.36 and 4.51×10^{-4} sec^{-1} at 80° and 97°, respectively, when 2 milliequivalents of catalyst was used in the transesterification reaction (see Table XX). At the higher catalyst concentration (4 milliequivalents) the corresponding values were 3.33 and 5.52×10^{-4} sec^{-1}, respectively (see Table XX). From these data the frequency factor (A) and activation energy (E) for the reaction were calculated from the Arrhenius equation (58). The latter can be expressed in the form

$$\log k_r = \log A - E/2.303RT \qquad (7)$$

where k_r is the rate constant for the reaction; R is the gas constant (1.987 $cal.deg.^{-1}$ $mole^{-1}$) and T is the absolute temperature. The above equation requires that the plot of log k_r against 1/T be linear; the slope being equal to -E/2.303R. The activation energy, at the 2 milliequivalent level of catalyst, was found to be 9.9 kcal $mole^{-1}$.

Since the transesterification reaction was found to follow first order kinetics in methyl myristate and sucrate ion, the pseudo first order rate constant 2.36×10^{-4} sec^{-1} had to be converted to the corresponding

second order rate constant before the frequency factor could be determined. Although the concentration of potassium sucrate in the reaction solution was 6.25×10^{-3} moles liter^{-1} (320 ml of the reaction solution contained 2 mM of potassium sucrate), the effective sucrate ion concentration was only 4.69×10^{-3} moles liter^{-1} since the potassium sucrate was only approximately 75% dissociated (see Figure VI). The second order rate constant was therefore found to be 5.03×10^{-2} liters moles^{-1} sec^{-1}. Substitution of the values for the activation energy (9.9 kcal mole^{-1}), absolute temperature (353°K) and the second order rate constant (5.03×10^{-2} liters moles^{-1} sec^{-1}) in equation 7 gave a value of approximately 0.7×10^{5} liters moles^{-1} sec^{-1} for the frequency factor.

The activation energy was calculated from the data at the higher catalyst concentration (4 milliequivalents) in the same way, and was found to be 7.7 kcal mole^{-1}. Since solubility characteristics of the catalyst were abnormal at 97° (see above) this value is undoubtedly too low.

<u>The thermodynamic constants of the transesterification reaction</u>

Since the activation energy (E) for the reaction,

Sucrose + Methyl myristate $\longrightarrow$ Sucrose myristate + Methanol $\uparrow$

was found to be 9.9 kcal mole^{-1} (see page 76), the entropy of activation ($\Delta S^{\ddagger}$) can be calculated from the equation,

$$k_r = e\, kT/h\, e^{\Delta S^{\ddagger}/R}\, e^{-E/RT} \qquad (8)$$

where k is the Boltzmann constant (1.380 x 10^{-16} erg deg.$^{-1}$ molecule^{-1}); h is Plancks' constant (6.624 x 10^{-27} erg sec.); k_r is the second order rate constant, 5.03 x 10^{-2} liters moles^{-1} sec^{-1} (see page 76), T is the absolute temperature (353° K) and R is the gas constant (59). The value for $\Delta S^{\ddagger}$ was found to be -38 entropy units (cal deg.$^{-1}$). The heat of activation $\Delta H^{\ddagger}$ (59) was calculated from the equation

$$E = \Delta H^{\ddagger} + RT \qquad (9)$$

and the free energy of activation (59) from the equation

$$\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\, \Delta S^{\ddagger} \qquad (10)$$

The approximate values of these thermodynamic constants are listed below,

$\Delta F^{\ddagger}$ = 22 kcal mole^{-1},
$\Delta H^{\ddagger}$ = 9 kcal mole^{-1},
$\Delta S^{\ddagger}$ = -38 entropy units.

The isolation of pure sucrose mono- and dimyristate

Sucrose (60 mM) was reacted with methyl myristate (20 mM), in the absence of n-octadecane, in anhydrous DMF (300 ml) using potassium methoxide (4 milliequivalents) in anhydrous methanol (0.680 ml) to form the catalyst. The standard reaction conditions in all other respects were used. No attempt was made to follow the rate of reaction. After a reaction period of 2-1/2 hours the reaction solution was taken to dryness at 70° on a rotary evaporator. The last traces of DMF was removed by azeotropic distillation with benzene. After the residue was dried under reduced pressure over phosphorous pentoxide, it was dissolved in n-butanol (250 ml). The n-butanol solution was then washed with two aliquots of 5% aqueous sodium chloride (200 ml per aliquot), and the combined washings were re-extracted with a further aliquot of n-butanol (100 ml). The combined n-butanol extracts were dried over anhydrous sodium sulphate (40 g). After standing overnight the n-butanol solution was filtered through anhydrous sodium sulphate (25 g); the sodium sulphate (65 g), which was collected on the filter, was washed thoroughly with n-butanol (100 ml). The n-butanol solution was then taken to dryness on a rotary evaporator and the residue gave a clear solution when it was redissolved in chloroform (50 ml).

The chloroform extracts from three identical experiments were combined and the solvent removed under reduced pressure. Subsequently the white residue of crude sucrose myristate was dried, under reduced pressure over phosphorous pentoxide, and weighed (26.57 g).

The crude sucrose myristate (26.57 g) was dissolved in chloroform (150 ml) and chromatographed on silicic acid (250 g, Mallinkrodt) using graded elution with methanol/chloroform solutions. As the chromatogram was developed the separation of constituents was clearly visible against a translucent background. The chromatogram only became opaque after 400 ml of 10% methanol/chloroform had passed through the column.

The separation was also followed by taking the infrared spectra of aliquots of the eluant. In this way it was possible to isolate three fractions, as shown in Table XXI, containing methyl myristate, sucrose dimyristate and sucrose monomyristate.

The sucrose monomyristate (17.60 g) was precipitated from acetone (100 ml) at -5° to yield pure sucrose monomyristate (15.60 g) as a white amorphous solid m.p. 180-186°; $[\alpha]_D$ + 36.7° (c, 4.8% in chloroform); saponification equivalent, 549 (theory 552). Anal. Calc. for $C_{26}H_{48}O_{12}$: C, 56.50%; H, 8.70%. Found: C, 56.36%;

TABLE XXI

Chromatographic separation of sucrose myristates on silicic acid

Fraction No.	Solvent	Volume of solvent (ml)	Weight of fraction (g)	Compound(1)
I	chloroform	2,000	0.80	methyl myristate
II	10% methanol/ chloroform	250	7.95	dimyristate
III	"	1,400	17.60	sucrose monomyristate
Total weight of sucrose myristates 25.55 (Theory 33.12)				

1. The residue from Fraction I was actually collected in the first 700 ml of eluant and its infrared spectrum was identical to that of an authentic sample of methyl myristate.

H, 8.63%. In contrast to the results of Billy (3) sucrose monomyristate dissolved readily in hot acetone with no precipitation of sucrose. The infrared spectrum of sucrose monomyristate (M.W. 552) is shown in Figure IX.

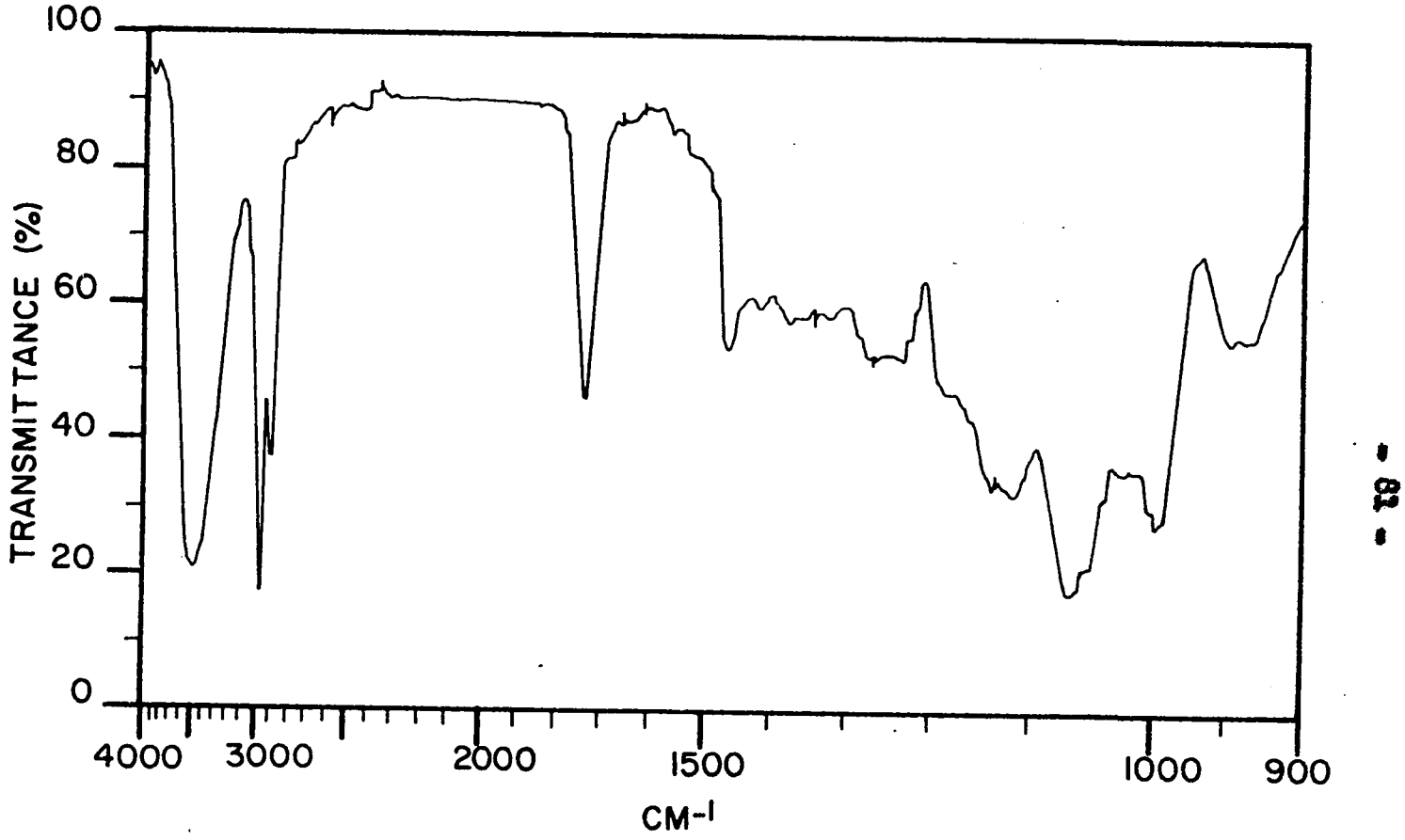


Figure IX. The infrared spectrum of sucrose monomyristate.

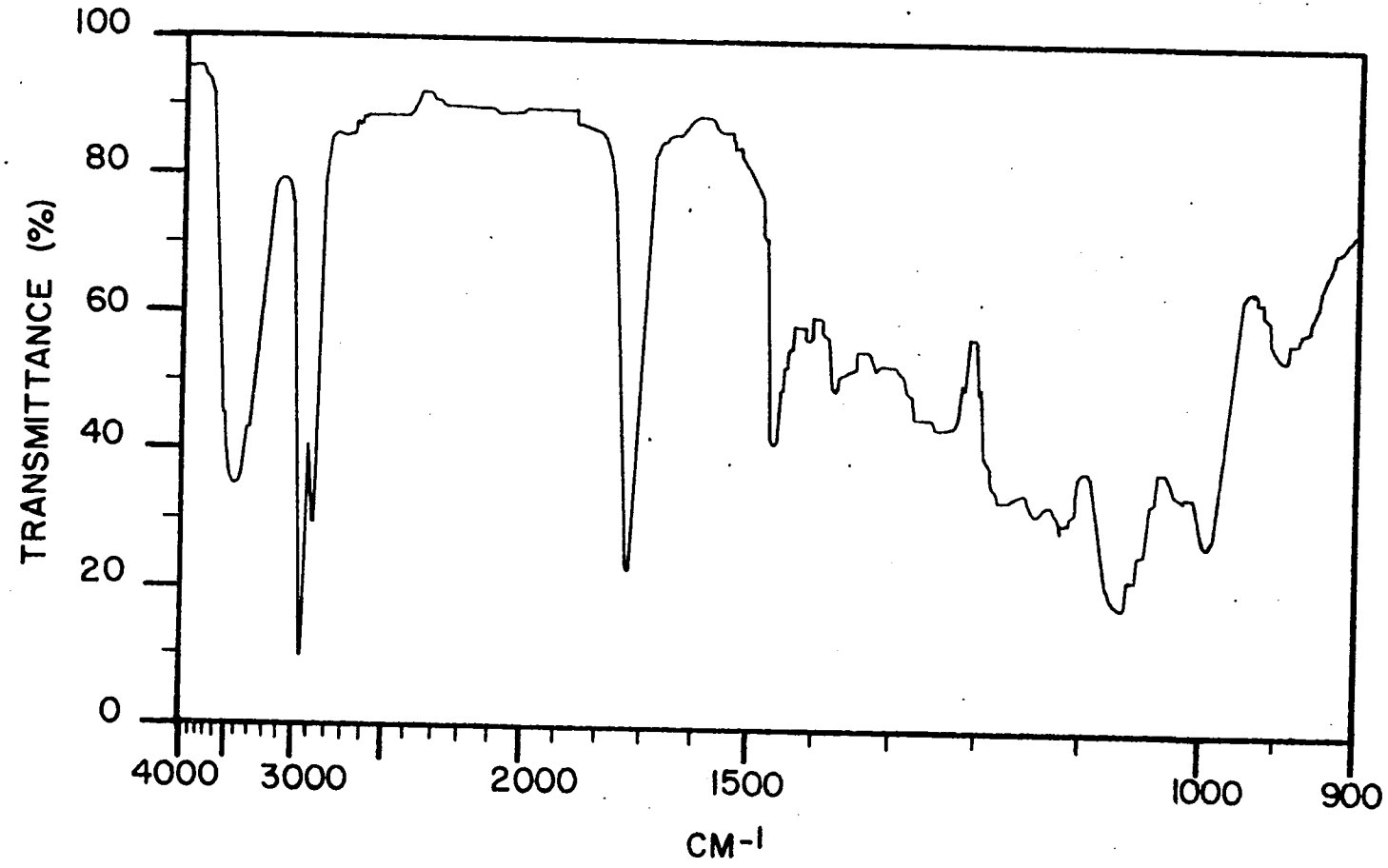


Figure X. The infrared spectrum of sucrose dimyristate.

The sucrose dimyristate (7.95 g) was precipitated first from methanol and then from acetone to yield sucrose dimyristate (6.05 g) as a white amorphous solid, m.p. 139-145°; $[\alpha]_D$ + 29.7° (c, 5.2% in chloroform); saponification equivalent 376 (theory 381). Anal. Calc. for $C_{40}H_{74}O_{13}$: C, 63.0%; H, 9.77%. Found: C, 63.39%; H, 9.91%. The latter values are slightly high and suggest the presence of a small amount of more highly substituted myristoyl esters of sucrose. The infrared spectrum of sucrose dimyristate (M.W. 762) is shown in Figure X.

Reaction equilibria

The equilibrium constant for the reaction,

Sucrose + Sucrose dimyristate $\rightleftharpoons$ 2 Sucrose monomyristate,

was determined at two different concentrations of sucrose.

Sucrose (1 mM) was dissolved in anhydrous DMF (15 ml) at 80° in an oil bath, and potassium methoxide solution (0.2 ml containing 0.2 milliequivalents of potassium) was added. Since potassium sucrate had a tendency to precipitate from solution under these conditions, methanol (5 ml) was added to obtain a homogeneous solution. The solution was then taken to dryness at 80° on a rotary evaporator, and the residue dried under reduced pressure over phosphorous pentoxide

for 2 hours. Pure sucrose monomyristate (1 mM) was then added to the dry residue of sucrose and potassium sucrate. The mixture was dissolved in anhydrous DMF (15 ml) and maintained at a temperature of 80° for 1-1/2 hours and at room temperature for a further 1 hour period. During the equilibration period the standard taper round bottom reaction flask (25 ml) was stoppered, care being taken to prevent any moisture reaching the reaction solution. After the reaction was complete the catalyst was neutralized by the addition of a DMF solution (1 ml) containing potassium hydrogen phthalate (0.2 milliequivalent). The resulting solution was taken to dryness at 80° on a rotary evaporator, and the residue dried once more under reduced pressure over phosphorous pentoxide. The residue was then transferred quantitatively to a separatory funnel with n-butanol (30 ml) and a 5% solution of sodium chloride (25 ml). After washing the butanol layer with a further aliquot of the sodium chloride solution (10 ml), the combined aqueous extracts were re-extracted with n-butanol (10 ml). The n-butanol extract was diluted with chloroform (100 ml) resulting in immediate precipitation of any sodium chloride in solution. After the solution was dried over anhydrous sodium sulphate, the solids were removed by filtration, and the clear filtrate was taken to dryness under reduced pressure. The residue of sucrose myristates

(466.9 mg) was obtained as a white amorphous powder. Chromatography of the latter on silicic acid (10 g) using graded elution with methanol/chloroform solutions enabled the concentrations of sucrose mono- and dimyristate to be determined. Fractions (5 ml) of the eluant from the chromatogram were collected, with an automatic collector, and the infrared spectrum of the sucrose ester in each fraction was compared with the spectra of pure sucrose mono- and dimyristate (see Figures IX and X). Seventeen fractions of 5% methanol/chloroform followed by ten fractions of 10% methanol/chloroform were collected. The first twenty fractions contained sucrose dimyristate (124.7 mg, 0.164 mM) and the last ten fractions contained sucrose monomyristate (336.9 mg, 0.61 mM). The two intermediate fractions contained no sucrose ester, indicating that a complete separation of sucrose monomyristate from sucrose dimyristate was achieved.

Another experiment, carried out under identical experimental conditions except that only 0.5 mM of sucrose was used initially, yielded sucrose myristates (434.0 mg). Chromatography of a sample of the latter (420.0 mg) on silicic acid, as described above, separated the sucrose dimyristate (152.0 mg, 0.199 mM) from the sucrose monomyristate (267.0 mg, 0.483 mM).

Since the infrared spectra of fractions of the eluant from the chromatograms did not reveal the presence of significant amounts of myristoyl esters of sucrose containing more than two myristoyl groups per sucrose molecule, as revealed by the relative intensity of the OH to the C=O stretching vibration (see Figures IX and X), the equilibrium constant K_1 could be calculated (see below) from equations 13 and 14. It was observed, however, that the concentration of the higher substituted sucrose myristates was larger in the experiment in which 0.5 mM of sucrose was used.

If in the transesterification reaction,

x = the initial concentration of methyl myristate,

y = " " " " sucrose,

z = the moles of methyl myristate which had reacted at equilibrium,

M = the moles of sucrose monomyristate at equilibrium,

then at equilibrium the concentrations of the compounds in the reaction would be

sucrose monomyristate = M,

sucrose dimyristate = $(z - M)/2$,

sucrose = $y-[M-(z-M)/2] = (2y-z-M)/2$

The equilibrium constant (K_1), therefore, could be expressed mathematically as

$$K_1 = \frac{[\text{sucrose monomyristate}]^2}{[\text{sucrose}][\text{sucrose dimyristate}]} = 4M^2/(2y-z-M)(z-M) \quad (11)$$

When it was assumed that all of the methyl myristate was converted to sucrose myristates, i.e. $z = x$, equation 11 reduced to

$$K_1 = 4M^2/[(2y-x-M)(x-M)] \quad (12)$$

If $y = 2$ and $x = 1$,

$$K_1 = [2M/(1-M)]^2/[2M/(1-M) + 3] \quad (13)$$

If $y = 1.5$ and $x = 1$,

$$K_1 = [2M/(1-M)]^2/[1/2[2M/(1-M)] + 2] \quad (14)$$

The equilibrium constant K_1 could be calculated from the molar ratio $2M/(1-M)$ of sucrose mono- and dimyristates. The molar ratios for the equilibrium reactions using 1 mM and 0.5 mM of sucrose, were 3.72 and 2.43, respectively. Substitution of these values in equations 13 and 14 respectively, gave values for K_1 of 2.0 and 1.8. In all subsequent calculations a value of $K_1 = 2$ was assumed since the error, introduced by ignoring the presence of higher substituted sucrose myristates, was less for this value.

It should be noted that y and x are the total moles of compound, whether in the free state or present as sucrose ester, at the beginning of a reaction.

The rate of equilibration of C^{14}-labelled sucrose with sucrose mono- and dimyristate

The rate of equilibration of C^{14}-labelled sucrose with sucrose mono- and dimyristate was studied by approaching the equilibrium from both sides.

Uniformly C^{14}-labelled sucrose (0.495 mM) was dissolved in anhydrous DMF (4 ml) and potassium methoxide (0.1 milliequivalents) in methanol (27.5 µl) was added. The solution was then taken to dryness in a rotary evaporator at 80° under reduced pressure. The residue of sucrose and potassium sucrate was subsequently dried under reduced pressure over phosphorous pentoxide. When the dry residue was dissolved in anhydrous DMF (5 ml) at 80°, the resulting DMF solution had a milky appearance undoubtedly due to the presence of undissolved potassium sucrate. A clear homogeneous solution, however, was obtained immediately when pure sucrose monomyristate (0.466 mM) was added to the solution. The reaction solution (5.5 ml) was maintained at 80° by means of a thermostatically controlled oil bath. Aliquots (1 ml) were taken at intervals of 10, 30, 45 and 65 minutes, and

the solvent removed rapidly (3 minutes) under reduced pressure. The residue was extracted with three aliquots of chloroform (2 ml), and the combined chloroform extract was transferred quantitatively to a small chromatographic column containing silicic acid (1 g) in chloroform. Elution with 10% methanol/chloroform (25 ml) followed by removal of the solvent, under reduced pressure on a rotary evaporator, yielded C^{14}-labelled sucrose myristates uncontaminated with C^{14}-labelled sucrose.

The specific activity of the C^{14}-labelled sucrose and sucrose myristates were determined using the apparatus and method described by Calvin (60) except that a 0.2 M solution of barium hydroxide was used instead of sodium hydroxide. A sample of the material to be analyzed was combusted in the presence of oxygen, and the carbon dioxide which formed was bubbled through the barium hydroxide solution. The barium carbonate which precipitated was collected on a tared filter paper and then washed successively with hot water, ethanol and ether. After drying overnight, under reduced pressure over phosphorous pentoxide, the weight of precipitate was determined by reweighing the filter paper. All samples were counted at infinite thickness and required 123.5 mg of barium carbonate for a planchet having an area of 4.94 cm^2. Under the experimental conditions used it was found that

mock combustions gave a negligible blank of less than 0.3 mg of barium carbonate. All specific activities were calculated from the time taken for 10^4 disintegrations. However, since the exact composition of the sucrose myristates was not determined, the specific activities were calculated as the number of counts per minute per milligramme of barium carbonate (c.p.m./mg $BaCO_3$). The C^{14}-labelled sucrose was found to have a specific activity of 8,475 c.p.m./mg $BaCO_3$. The results for equilibration of C^{14}-labelled sucrose and sucrose monomyristate is given in Table XXII.

Another equilibration experiment using sucrose dimyristate (0.475 mM) and C^{14}-labelled sucrose (0.505 mM) in the presence of potassium sucrate (0.1 milliequivalent) was carried out under the same experimental conditions described above. The results are given in Table XXII.

The chromatographic method for isolating the C^{14}-labelled sucrose myristates was established by taking synthetic mixtures of sucrose monomyristate and sucrose dimyristate with C^{14}-labelled sucrose in anhydrous DMF, but in the absence of potassium sucrate, and recovering the corresponding sucrose ester, as described above, in quantitative yield and uncontaminated with C^{14}-labelled sucrose.

The theoretical specific activity (c.p.m./mg $BaCO_3$) of the sucrose myristates, isolated after equilibration, were calculated in the following manner. The concentrations of sucrose mono-(M) and dimyristate (0.233 - M/2) were calculated by substituting the values for y, x and K_1 in equation 12. For example these values were 0.961, 0.466 and 2, respectively, for the experiment using C^{14}-labelled sucrose (0.495 mM) and sucrose monomyristate (0.466 mM). The calculated equilibrium concentrations of sucrose mono- and dimyristate were found to be 0.39 mM and 0.038 mM, respectively. The theoretical specific activity was then calculated from equation 15.

$$A = 12fSS^*/(d+m) \qquad (15)$$

where A = the theoretical specific activity,

S^* = the specific activity of the original C^{14}-labelled sucrose,

S = the total moles of sucrose in the sucrose mono- and dimyristate,

f = the fraction of the total moles of sucrose in the original reaction solution, whether present as sucrose myristate or free sucrose which was C^{14}-labelled,

d = the moles of barium carbonate which would be obtained on combustion of the sucrose dimyristate,

m = the moles of barium carbonate which would be obtained on combustion of the sucrose mono-myristate.

The values for d, m, f, S and S* in the above experiment were 1.52 (mM), 10.14 (mM), 0.5236, 0.428 (mM) and 8,475, respectively. Substitution of these values in equation 15 gave a value for the theoretical specific activity (A), assuming random distribution of the sucrose molecules in the sucrose myristate of 1,950 c.p.m./mg $BaCO_3$.

A similar calculation for the experiment using C^{14}-labelled sucrose (0.505 mM) and sucrose dimyristate (0.475 mM) gave values for d, m, f, S and S* of 11.0 (mM), 10.40 (mM), 0.515, 0.675 (mM) and 8,475, respectively, and a value of 1,650 c.p.m./mg $BaCO_3$ for the theoretical specific activity (A). The sucrose mono- and dimyristate concentrations at equilibrium were calculated to be 0.4 mM and 0.275 mM, respectively.

The theoretical weight yields of the C^{14}-labelled sucrose myristates in 1 ml of reaction solution were calculated, assuming complete equilibration of the C^{14}-labelled sucrose and sucrose ester, from the calculated equilibrium concentrations of C^{14}-labelled sucrose mono- and dimyristate. The calculated yields were 44.4 and 78.1 mg/ml as compared with the highest experimental yields, after 65 minutes reaction, of 38.3 and 72.2 mg/ml, respectively (see Table XXII).

TABLE XXII

Equilibration of C^{14}-labelled sucrose with sucrose monomyristate and sucrose dimyristate at 80°

Reaction time (minutes)	Sucrose monomyristate(1) Yield of sucrose myristates (mg)	Sucrose monomyristate(1) Specific activity of sucrose myristates c.p.m./mg $BaCO_3$	Sucrose dimyristate(2) Yield of sucrose myristates (mg)	Sucrose dimyristate(2) Specific activity of sucrose myristates c.p.m./mg $BaCO_3$
10	37.1	1,216	71.7	1,337
30	37.5	1,404	70.7	1,418
45	38.3	1,432	70.3	1,437
65	38.3	1,471	72.2	1,442
Theoretical specific activity		1,950		1,650

1. Reaction carried out using sucrose monomyristate and C^{14}-labelled sucrose as reactants.
2. Reaction carried out using sucrose dimyristate and C^{14}-labelled sucrose as reactants.

The effect of methanol on the rate of transesterification

An approximate value for the equilibrium constant for the reaction,

Sucrose + Methyl myristate $\rightleftharpoons$ Sucrose myristates + Methanol,

was calculated from the data obtained by studying the effect of methanol on the rate of the standard transesterification reaction (see Table XXIII). A saturated methanolic solution (0.68 ml) of potassium methoxide (4 milliequivalents) was used to catalyse the reaction. Two aliquots of the reaction solution were taken at intervals of 5, 10, 20, 40, 80 and 120 minutes. The percentage of methyl myristate which had reacted in one aliquot (0.6 ml) was determined immediately by G.L.P.C. using n-octadecane as an internal standard. The other aliquot (2.0 ml) was transferred to a small stoppered test tube (total volume 2.2 ml) and allowed to equilibrate at 80° for 145, 140, 130, 110, 70 and 30 minutes, respectively. At the end of the equilibration period the percentage of methyl myristate which had reacted was determined once more. Care was taken at all times to exclude moisture from the reaction solutions. From the data in Table XXIII it was possible to calculate the equilibrium constant K_2 for the above reaction.

The concentrations of the compounds in reaction at equilibrium would be,

	Sucrose myristates	= M + (z-M)/2	moles,
	Methanol	= R	"
	Sucrose	= (2y-z-M)/2	"
and	Methyl myristate	= x - z	"

where y and x were the initial concentrations of sucrose and methyl myristate, respectively; z was the concentration of methyl myristate which had reacted at equilibrium and M was the equilibrium concentration of sucrose monomyristate. The equilibrium constant could therefore be expressed mathematically as,

$$K_2 = [\text{Sucrose myristates}][\text{Methanol}]/[\text{Sucrose}][\text{Methyl myristate}]$$
$$= R(M + z)/[(2y-z-M)(x-z)] \qquad (16)$$

If it was assumed that only sucrose monomyristate was formed during the reaction then M = z and equation 16 reduced to

$$K_3 = MR/[(y-M)(x-M)] = zR/[(y-z)(x-z)] \qquad (17)$$

When a value of 2 was assumed for the equilibrium constant K_1, the value of M could be calculated from the known values for y (60 mM) and z (see Table XXIV) using equation 13 (see page 87). The value of R was calculated from the value of x (20 mM) and the data in Table XXIII. For example, when a sample which had reacted to the extent of 11.0% in

5 minutes was allowed to equilibrate for a further 145 minutes only 55.4% of the methyl myristate had reacted. The concentration of methanol at equilibrium was therefore 20(0.554-0.11) = 8.88 mM (see Tables XXIII and XXIV). Substitution of y, x, z, M and R in equations 16 and 17 gave the values for K_2 and K_3 shown in Table XXIV.

TABLE XXIII

Effect of methanol concentration on the extent of reaction at 80°

Time (minutes)	5	10	20	40	80	120	150
% methyl myristate reacted	11.0	24.7	44.2	65.8	84.2	90.2	94.5
Time (minutes) for the reaction to reach equilibrium	145	140	130	110	70	30	
% methyl myristate reacted	55.4	62.2	69.8	78.1	90.0	94.5	

TABLE XXIV

The equilibrium constants K_2 and K_3

Time (minutes) for the reaction to reach equilibrium	M (mM)	R (mM)	$\dot{z}$ (mM)	K_2	K_3
145	9.3	(0.554-0.11)20 = 8.88	0.554 x 20 = 11.08	0.204	0.225
140	10.81	(0.622-0.247)20= 7.50	0.622 x 20 = 12.44	0.239	0.260
130	11.27	(0.698-0.442)20= 5.12	0.698 x 20 = 13.96	0.226	0.257
110	12.30	(0.781-0.658)20= 2.46	0.781 x 20 = 15.62	0.171	0.198
70	13.75	(0.90 - 0.842)20=1.16	0.90 x 20 = 18.00	0.209	0.248
30	14.25	(0.945-0.902)20= 0.86	0.945 x 20 = 18.90	0.316	0.359
		Average value for the equilibrium constant		0.227	0.258

Determination of the equilibrium constant K_2 starting with sucrose and methyl myristate

A standard transesterification solution containing sucrose (60 mM) methyl myristate (20 mM) and n-octadecane (12.75 mM) in anhydrous DMF (300 ml) was prepared. An aliquot (17 ml) of the latter, containing sucrose (3.18 mM) and methyl myristate (1.06 mM) was transferred to a test tube (total capacity 17.2 ml), stoppered with a rubber serum cap, with a calibrated hypodermic syringe. Methanol (1 mM) containing potassium methoxide (0.116 mM) was then added to the reaction solution through the serum cap with a calibrated micro syringe (total volume 0.1 ml). The reaction solution was maintained at constant temperature of 80° with an oil bath. The rate of equilibration was determined by taking aliquots (0.1 ml) of the reaction solution, periodically, and analyzing for methyl myristate by G.L.P.C., using n-octadecane as an internal standard. It was assumed that the equilibrium condition had been reached when there was no further decrease in the concentration of methyl myristate (see Table XXV). The rate at which the reaction approaches the equilibrium condition is shown graphically in Figure XI.

At the end of the equilibration period methanol (10 mM) was added to the reaction solution. When the latter was analyzed for methyl myristate (see above) after 30 minutes it was found that the concentration of methyl myristate had increased significantly (see Table XXV). This established that the catalyst was not destroyed during the equilibration period. It should be noted that care was taken to exclude moisture from the reaction solution at all times.

If a value of 2 was assumed for K_1 in equation 11 the sucrose monomyristate concentration (M = 0.468 mM) could be calculated (see page 87) from the initial concentration of sucrose (y = 3.18 mM) and the concentration of methyl myristate (z = 0.549 mM) which had reacted at equilibrium. Substitution of the values for y (3.18 mM), x (1.06 mM), M (0.468 mM), R (1.549 mM) and z (0.549 mM) in equation 16 (see page 95) gave a value for K_2 of 0.577.

Determination of the equilibrium constant K_2 starting with sucrose monomyristate and methanol

Anhydrous DMF (100 ml) was refluxed at 97° at 140 mm pressure with nitrogen passing through the solution for 1 hour. An aliquot (15 ml) was transferred to a test tube, through a rubber serum cap, with a calibrated hypodermic

syringe. The test tube contained weighed amounts of pure sucrose monomyristate (1 mM) and n-octadecane (0.638 mM). The reaction solution was maintained at a constant temperature of 80° in an oil bath. After 15 minutes methanol (1 mM) containing potassium methoxide (0.116 mM) was added to the reaction solution, through the serum cap, with a calibratedmicro syringe. The rate of equilibration was followed by analyzing aliquots (0.1 ml) of the reaction mixture by G.L.P.C. for methyl myristate, using n-octadecane as an internal standard. It was assumed that equilibrium had been reached when there was no further increase in the concentration of methyl myristate (see Table XXV). At the end of the equilibration period the presence of catalyst was confirmed by the addition of methanol as described previously (see page 99). The rate at which the reaction approaches equilibrium is shown graphically in Figure XI.

If a value of 2 was assumed for K_1 in equation 11 the sucrose monomyristate concentration (M = 0.312 mM) could be calculated (see page 87) from the initial concentration of sucrose (y = 1 mM) and the concentration of methyl myristate (z = 0.472) which had reacted at equilibrium. Substitution of the values for y (1 mM), x (1 mM), z (0.472 mM), M (0.31 mM) and R (0.472 mM) in

equation 16 (see page 87) gave a value for K_2 of 0.576.

TABLE XXV

The rate of equilibration at 80°

Percentage Reaction

Time (minutes)	Reactants: Sucrose + methyl myristate	Reactants: Sucrose monomyristate methanol
15		34.5
30	34.4	42.8
60	44.9	48.5
90	49.7	49.9
120	50.7	
125		50.5
165		52.4
240	51.8	
885		52.8
960	51.8	
	after addition of methanol (10 mM)	
30	>>51.8	>>52.8

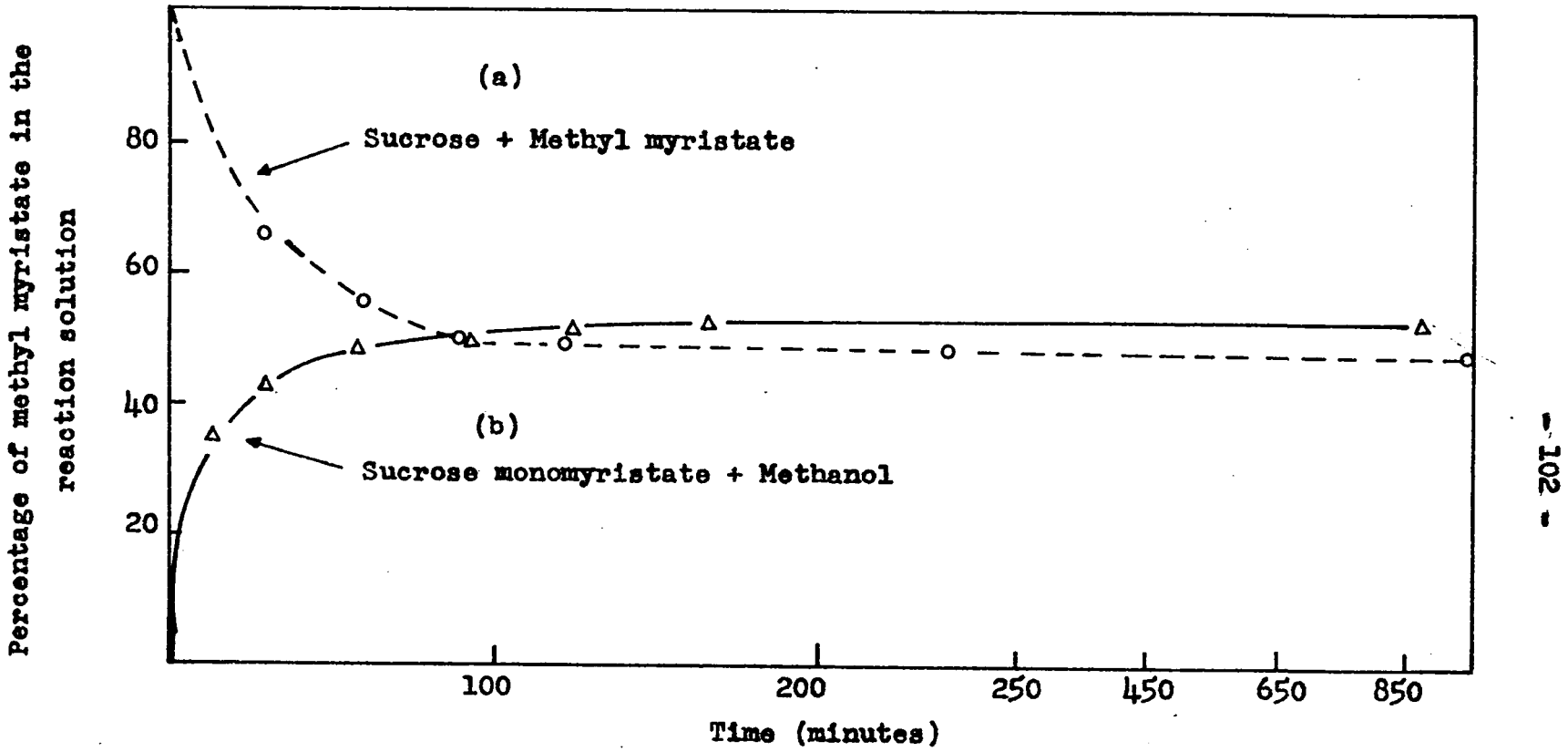


Figure XI. The rate of equilibration of sucrose and methyl myristate with sucrose monomyristate and methanol in N,N-dimethylformamide solution at 80°. In (a) the reaction solution was 18.75, 6.25, 6.25 and 0.7 x 10^{-2} molar in sucrose, methyl myristate, methanol and alkoxide ion, respectively, whereas in (b) the solution was 6.25, 6.25 and 0.7 x 10^{-2} molar in sucrose monomyristate, methanol and alkoxide ion, respectively.

The effect of the relative concentrations of sucrose and methyl myristate on the yield of sucrose monomyristate

The yield of sucrose monomyristate (M) for any initial concentration of sucrose (y) and methyl myristate (x) was calculated by expressing equation 14 (see page 87) as,

$$M^2(1-4/K_1)-2yM-2yx-x^2 = 0 \qquad (18)$$

This expression assumed that the methyl myristate was converted quantitatively to sucrose mono- and dimyristates. Using a value of 2 for K_1, the above quadratic equation gave two solutions for M only one of which was positive, namely

$$M = \sqrt{y^2 + 2yx-x^2} - y \qquad (19)$$

If y and M were expressed as multiples of x (i.e. x = 1), equation 19 reduced to,

$$M = \sqrt{y^2 + 2y-1} - y \qquad (20)$$

Since equation 20 was satisfied by all values of y from 0.5 to ∞, the effect of sucrose concentration on the yield of sucrose monomyristate could be shown graphically by plotting M/x versus y/x. It should be noted, however, that equation 20 is only valid when an equilibrium exists exclusively between sucrose, sucrose dimyristate and

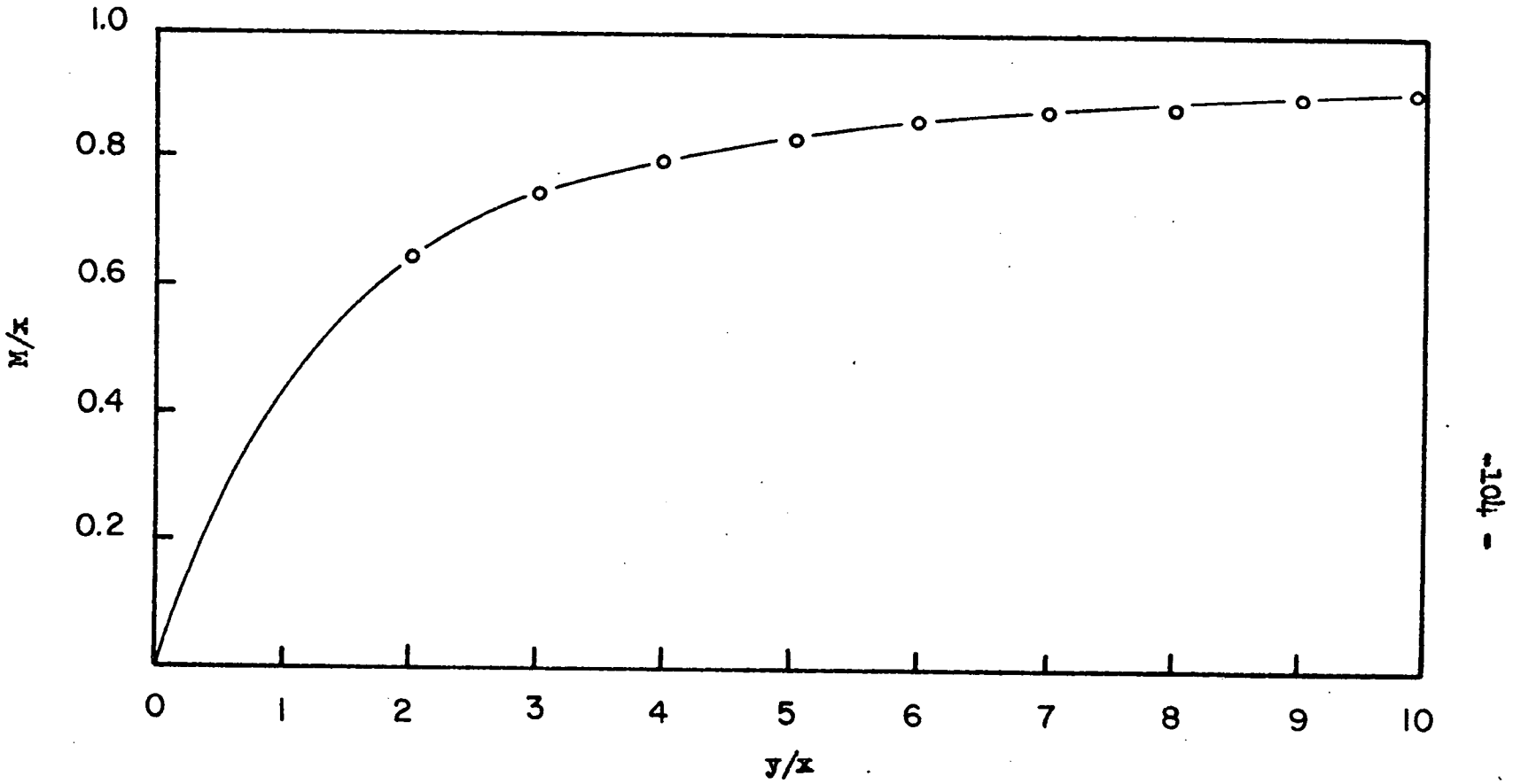


Figure XII. The plot of the calculated yield of sucrose monomyristate (in moles) per mole of methyl myristate (M/x), in the standard transesterification reaction at 80°, against the initial concentration of sucrose per mole of methyl myristate (y/x).

sucrose monomyristate. At concentrations of sucrose which are low relative to myristoyl group content substantial amounts of higher substituted myristoyl esters of sucrose will be formed in their equilibrium proportions. Therefore, equation 20 can only be given values for $y/x \geq 2$ for which the concentration of sucrose esters with more than two myristoyl groups is not significant (see page 86). Furthermore the plot of M/x against y/x must necessarily pass through the origin.

In Figure XII, M/x was plotted against y/x for all values of y from 2 to 10, and M from 0 to 1.0, and the resulting curve was extrapolated to pass through the origin.

Side reactions in the transesterification reaction

Two experiments were carried out to determine if any side reactions occurred during the transesterification reaction which could effect the rate of reaction, and the yield of sucrose ester.

In the first experiment a solution of sucrose (60 mM), methyl myristate (20 mM) and n-octadecane (12.75 mM) in anhydrous DMF (300 ml) was maintained at 80° and 75 mm pressure for 6 hours, with a stream of nitrogen passing through the solution. No catalyst was added to the reaction mixture. Aliquots of the reaction solution

were analyzed by G.L.P.C. periodically to determine the methyl myristate content, using n-octadecane as an internal standard. No decrease in concentration of methyl myristate was observed during the 6 hour reaction period.

In the second experiment the reaction solution contained methyl myristate (20 mM), n-octadecane (12.75 mM) and potassium methoxide (4 milliequivalents of potassium in 5 ml of anhydrous methanol). The experimental conditions and analytical methods were identical to those described above. Once again, no decrease in the methyl myristate content was observed over the 6 hour reaction period.

The preparation of sucrose myristates on a preparative scale

As a consequence of the kinetic studies of the reaction the following preparative procedure was developed.

Sucrose (60 mM) was dissolved in anhydrous DMF at 80° and 65 mm pressure with vigorous and continuous stirring, and a stream of nitrogen passing through the solution. After about 15 minutes the sucrose was completely dissolved and the solvent refluxed to a point in the Vigreaux column about two inches from the neck of the flask. Sodium methoxide solution (4 ml containing

4 milliequivalents) was added below the surface of the solution using a hypodermic syringe, in the usual manner. The evolution of methanol was rapid but caused no difficulties. After equilibrium was re-established, the oil bath temperature was raised to 100° whereupon the solution achieved a temperature of 80° and refluxed about half-way up the Vigreaux column. The still-head temperature was about 50°. The system was kept under these conditions for a further 15 minutes and then the methyl myristate (20 mM) was added using a hypodermic syringe. The solution was then maintained at 80° for 150 minutes. During this reaction period approximately 50 ml of the DMF distilled over slowly.

At the end of the reaction period, glacial acetic acid (0.5 ml) was added to the stirred reaction solution, at atmospheric pressure, to stop the reaction. The solution was then taken to dryness at 70°, under reduced pressure, in 30 minutes. The residue was partitioned between n-butanol (150 ml) and 5% sodium chloride (100 ml). The n-butanol phase was washed with a further aliquot of salt solution (50 ml). After re-extracting the combined aqueous phase with n-butanol (50 ml) the combined n-butanol phase was filtered through paper wetted with n-butanol. The filtrate was taken to dryness and the residue was heated with 100 ml of chloroform. Any

insoluble material was removed by filtration and the chloroform solution was taken to dryness. The residue of sucrose myristate (9.6 g) was then precipitated from acetone to yield sucrose myristates (9.2 g), as a white powder. (theory for pure sucrose monomyristate is 11.0 g).

The Structure of Sucrose Monomyristate

The tosylation of sucrose monomyristate

Pure sucrose monomyristate (0.6023 g) was reacted with p-toluenesulphonyl chloride (2.80 g) in anhydrous pyridine (20 ml) at room temperature for 12 hours. The excess p-toluenesulphonyl chloride was then destroyed by adding water (1 ml) to the reaction solution. After the latter was left standing at room temperature for 30 minutes, it was poured into ice water (50 ml) and extracted with five aliquots (20 ml) of chloroform. After washing the latter with 2N HCl, sodium bicarbonate solution (20% w/v) and finally with distilled water, the chloroform solution was dried by passing it through a filter paper. Removal of the chloroform, under reduced pressure, yielded a product which analyzed 12.28% S, for a sucrose 5.18-tosylate (1.48 g).

Iodination of sucrose 5.18-tosylate

Sucrose 5.18-tosylate (0.5396 g) was reacted with sodium iodide (0.6 g) in acetone (6 ml) in a sealed glass tube at 105° for 15 hours. After the reaction was complete, the glass tube was opened carefully and the sodium p-toluene sulphonate (0.1424 g) which had precipitated from the solution was collected on a tared Gooch crucible. The filtrate was taken to dryness and the residue was extracted with three aliquots (15 ml) of chloroform. After removing any free iodine and sodium iodide with sodium thiosulphate solution (10% w/v) and distilled water, the chloroform solution was dried by passing it through a filter paper. Removal of the chloroform, under reduced pressure, yielded sucrose 1.84 iodo, 3.34-tosylate (0.4474 g). Anal. Calc. for sucrose 1.84 iodo, 3.34-tosylate: S, 8.43%, I, 18.42%. Found: S, 8.30%; I, 18.90%.

Methylation of sucrose monomyristate

Pure sucrose monomyristate (2.03 g) was methylated, by the method of Kuhn (61, 62), in anhydrous DMF (60 ml) using methyl iodide (60 ml) and freshly prepared silver oxide (22 g). Finely divided anhydrous calcium sulphate (20 g) was added to the reaction

solution to ensure anhydrous conditions for methylation, and to reduce the possibility of ester hydrolysis. The reaction was assisted by vigorous agitation of the reaction solution, on a mechanical shaker, for 15 hours. At the end of the reaction period the DMF solution was filtered, and the clear filtrate diluted with chloroform (150 ml) to precipitate the silver iodide in solution. After the silver iodide was removed by filtration, the chloroform/DMF solution was taken to dryness on a rotary evaporator and the residue dried under reduced pressure over phosphorous pentoxide. The crude methylated sucrose ester (2.39 g) was obtained in quantitative yield. Since G.L.P.C. analysis revealed the presence of methyl myristate in the methylated sucrose monomyristate, a sample (2.35 g) was chromatographed on silicic acid (40 g) using graded elution with methanol/chloroform solutions as shown in Table XXVI. Fraction I was shown to be pure methyl myristate (1.9% of the total weight of the sample) since its infrared spectrum was identical with that of an authentic sample of methyl myristate. Fraction 2 was not investigated further although its infrared spectrum was identical with that of fractions 3 and 4. The latter (2.1894 g, 91.6% theory) were combined since their infrared spectra showed no absorption in the O-H stretching region, and was consistent

with that expected for hepta-O-methyl sucrose monomyristate.

TABLE XXVI

Chromatography of methylated sucrose monomyristate on silicic acid

Fraction	Solvent	Volume (ml)	Weight of residue (g)
1	chloroform	150	0.0442 (1)
2	"	100	0.1068
3	"	225	1.4985
4	10% methanol/ chloroform	200	0.6909
5	methanol	150	0.0030
	Total weight recovered		2.3434

1. This material was collected in the first 100 ml of chloroform.

Preparation of hepta-O-methyl sucrose

A sample (1.1200 g) of hepta-O-methyl sucrose monomyristate was transesterified with anhydrous methanol (10 ml) under reflux for 5 hours, using potassium methoxide (2 mM) as catalyst. At the end of the reaction period the catalyst was converted to potassium carbonate, by the addition of solid carbon dioxide, and the methanol

removed under reduced pressure. The residue was extracted with chloroform (50 ml) which was taken to dryness under reduced pressure. The residue was chromatographed on silicic acid (10 g) using graded elution with methanol/chloroform solutions. Elution with chloroform (100 ml) followed by removal of the solvent, yielded an oil (0.3958 g; 98.0% theory) the infrared spectrum of which was identical to that of methyl myristate. Further elution with 5% methanol/chloroform (100 ml) yielded hepta-O-methyl sucrose as an oil (0.6958; 92% theory). Anal. Calc. for $C_{19}H_{36}O_{11}$: methoxyl content, 49.3%. Found: 48.4%. The infrared spectrum of the latter in carbon tetrachloride solution (0.0025M) showed absorption, in the O-H stretching region, at 3485 cm^{-1}. The n.m.r. spectrum of the hepta-O-methyl sucrose in carbon tetrachloride (20% w/v) gave two doublets with τ values (64) of 4.73 and 4.53, and a common coupling constant of 3.5 c.p.s., for the anomeric hydrogen (30, 63). The relative intensities of the two doublets was 0.65 to 0.35; the doublet at lowest field (τ = 4.53) was more intense (see Figure XIII).

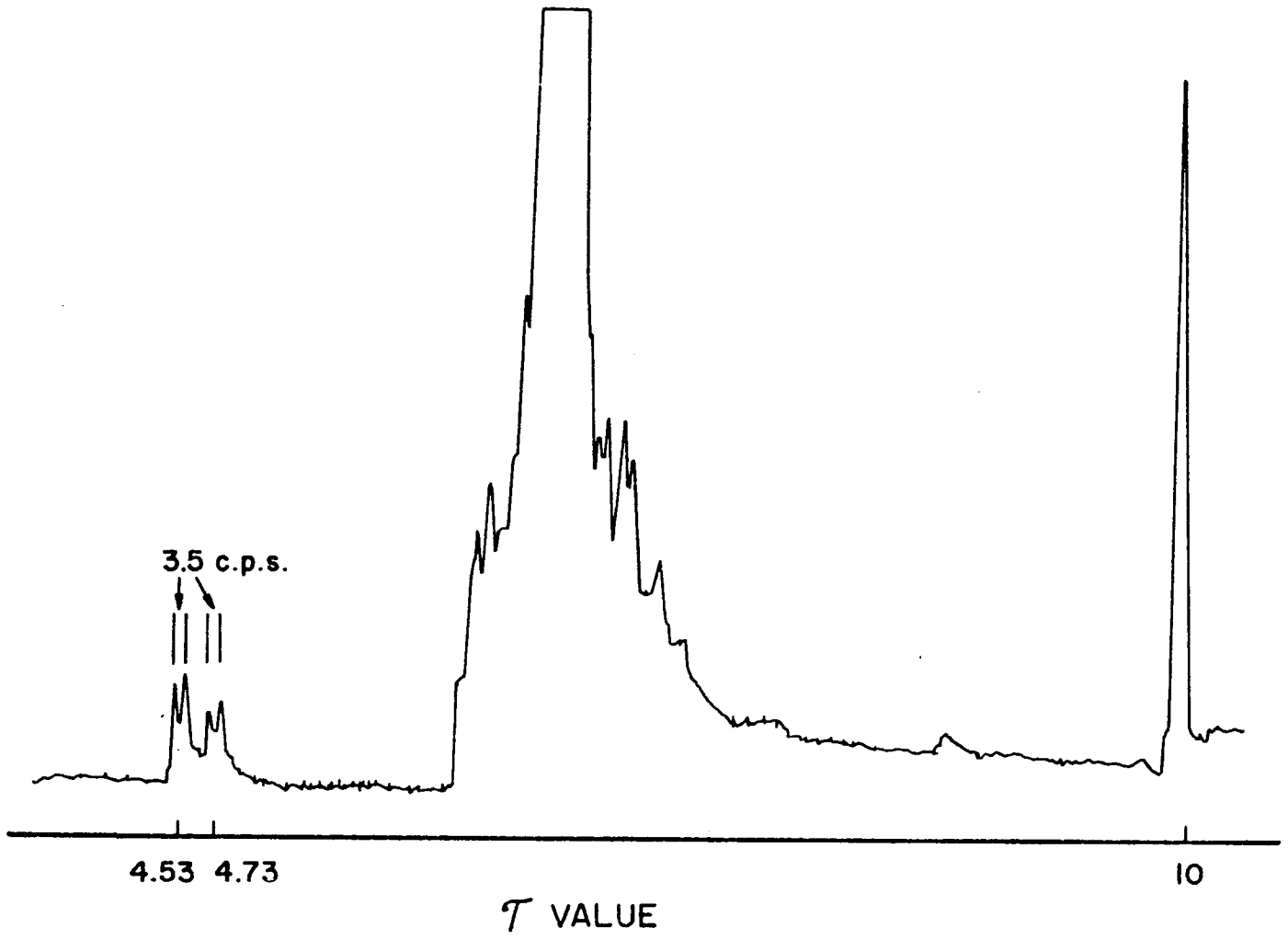


Figure XIII. The proton magnetic resonance spectrum of the mixture of hepta-O-methyl sucroses.

Tosylation of hepta-O-methyl sucrose

Hepta-O-methyl sucrose (0.1566 g) was reacted with p-toluene sulphonyl chloride (0.27 g) in anhydrous pyridine (2 ml) for 12 hours. At the end of the reaction the product was isolated as described previously (see page 108), to yield hepta-O-methyl sucrose monotosylate (0.2172 g) in quantitative yield. Anal. Calc. for $C_{26}H_{42}SO_{13}$: S, 5.39%. Found: S, 5.15%. The infrared spectrum of the latter in chloroform (5% w/v), was consistent with that expected for a tosyl ester of a methylated sugar. The n.m.r. spectrum in carbon tetrachloride (20% w/v) gave a quartet, with an average τ value of 2.55, and an intensity corresponding to four protons, for the aromatic proton (64). Two doublets, with a combined intensity corresponding to one proton, were also obtained once more for the anomeric hydrogen (see Figure XIV). The intensity ratio for the two doublets was 6.5:3.5; the signal at lowest field (τ = 4.53) having the greater intensity.

The preparation of deoxy hepta-O-methyl sucrose from hepta-O-methyl sucrose monotosylate

The hepta-O-methyl sucrose monotosylate (0.1019 g) was reacted with sodium iodide (0.6 g) in acetone (6 ml) at 105° for 15 hours in a sealed tube. At the end of the

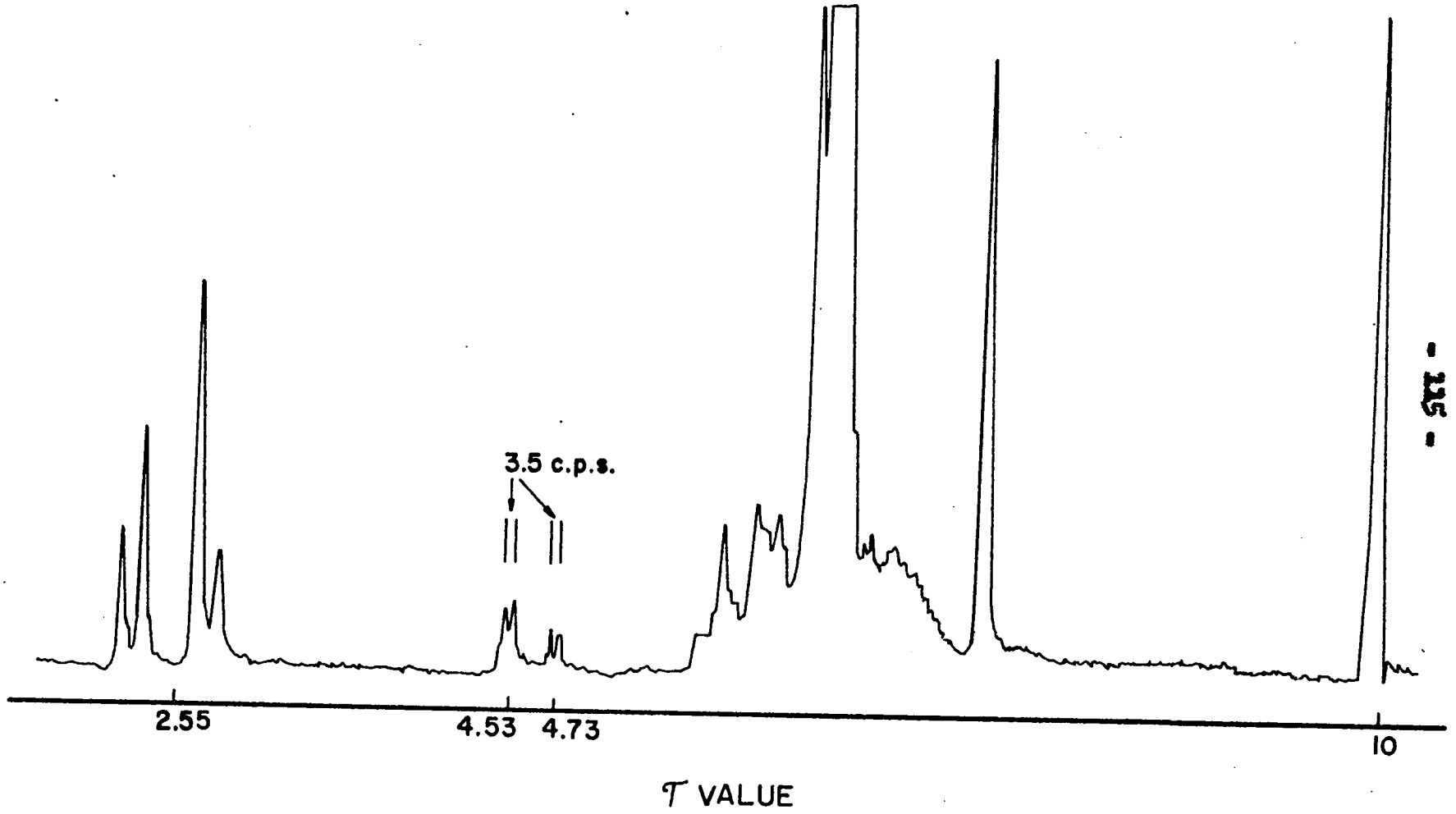


Figure XIV. The proton magnetic resonance spectrum of the mixture of the mono-O-tosyl hepta-O-methyl sucroses.

reaction period an oil (0.0916 g; 97.2% theory) hepta-O-methyl iodo-deoxy sucrose was isolated as described previously (see page 109). The n.m.r. spectrum showed that only 10% of the tosyloxy groups had not reacted with sodium iodide. The hepta-O-methyl iodo-deoxy sucrose was dissolved in methanol (6 ml) containing diethylamine (0.024 g) and reduced with hydrogen for 4 hours using palladium on charcoal as catalyst. The latter was then removed by filtration, and the methanol solution taken to dryness under reduced pressure, and the residue (0.061 g, 87% theory), hepta-O-methyl deoxy sucrose, was weighed. Anal Calc. for $C_{19}H_{36}O_{10}$: C-CH_3, 6.37%. Found 5.80%. The n.m.r. spectrum of the latter in carbon tetrachloride (20% w/v) showed that the aromatic protons (τ = 2.55) (64) had an intensity corresponding to only 0.4 protons, and the anomeric hydrogen was present as an ill-defined quartet with an average τ value of 4.72. Two doublets, however, were present for the protons of methyl groups (64) at τ values of 8.80 and 8.86, respectively, and had a common coupling constant of 6.1 c.p.s. The total intensity of the two doublets corresponded to 2.8 protons (theory 3 protons) and the ratio of the intensities of the doublets was 0.65:0.35. The signal at highest yield had the greater intensity (see Figure XV).

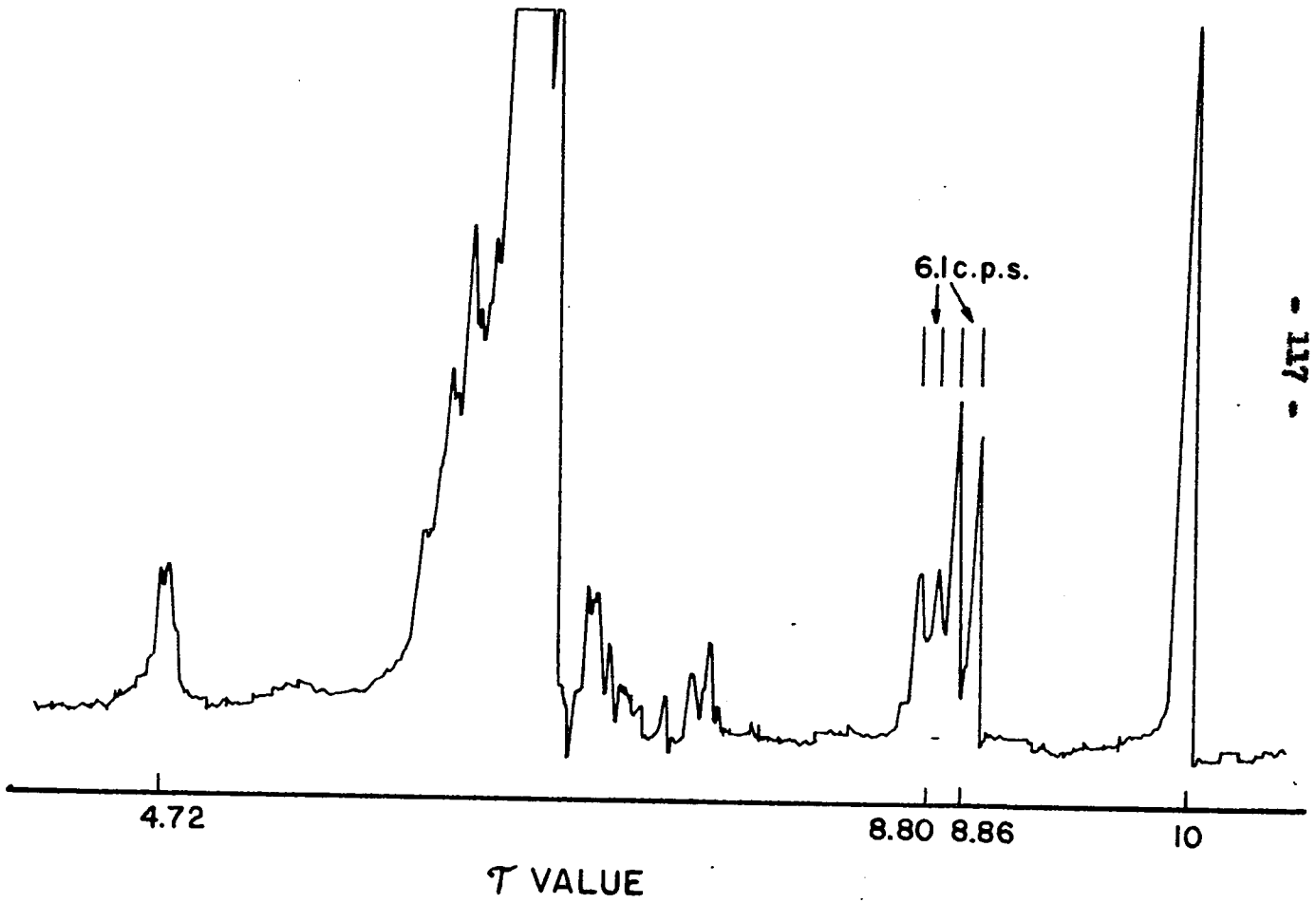


Figure XV. The proton magnetic resonance spectrum of the mixture of deoxy hepta-O-methyl sucroses.

Chromatography of hydrolysis and methanolysis products of hepta-O-methyl sucrose

The method of Hayward (21) was used for the hydrolysis of hepta-O-methyl sucrose (10 mg) with 0.05N H_2SO_4 (1 ml) on a steam bath for 2 hours. At the end of the reaction period, the H_2SO_4 was neutralized with solid barium carbonate, and the aqueous solution of the hydrolysis products was centrifuged. Aliquots of the supernatant solution was spotted on Schleicher and Schuell, orange ribbon 289, chromatographic paper. The chromatogram was developed with n-butanol-ethanol-water 5:1:4 (65) for 18 hours, using 2,3,4,6-tetra-O-methyl glucose and 2,3,4-tri-O-methyl glucose as standards. Two spray reagents were used, to detect the free sugars, namely 3% p-anisidine hydrochloride in moist n-butanol and 3% resorcinol in n-butanol containing HCl (65). The former spray reagent was used to detect both ketoses and aldoses whereas the latter was specific for ketoses (65). All Rf values were calculated relative to that of 2,3,4,6-tetra-O-methyl glucose (R_{TG}). Only three spots were present, with R_{TG} values of 0.81, 0.89 and 1.00, when the p-anisidine hydrochloride spray was used, and two spots were present, with R_{TG} values of 0.89 and 1.00, when the resorcinol spray was used (see Table XXVII). The R_{TG}

TABLE XXVII

Paper chromatography of hydrolysis products of hepta-O-methyl sucrose

Compound	Colour of spot	Spray reagent: p-anisidine hydrochloride R_{TG}	Spray reagent: Resorcinol R_{TG}
2,3,4-tri-O-methyl glucose(1)	red-brown	0.81	
1,3,4-tri-O-methyl fructose	yellow	0.89	
	pink		0.89
2,3,4,6-tetra-O-methyl glucose(1)	red-brown	1.00	
1,3,4,6-tetra-O-methyl fructose	pink		1.00

1. These compounds were used as standards.

values were in good agreement with the values reported (66) for 2,3,4-tri-O-methyl glucose (R_{TG} 0.83), 1,3,4-tri-O-methyl fructose (R_{TG} 0.85), 2,3,4,6-tetra-O-methyl glucose (R_{TG} 1.01) and 1,3,4,6-tetra-O-methyl fructose (R_{TG} 1.01).

Hepta-O-methyl sucrose (13.2 mg) was reacted with 3% methanolic HCl (0.20 ml) for 5 hours at room temperature. Aliquots of the reaction mixture (0.1 µl) was analyzed by G.L.P.C. on a butanediol succinate polyester column (57) at 160°, using a flow rate of 60 ml of argon per minute. The analysis showed the presence of ten methyl glycosides. The retention volumes of these compounds were calculated relative to that of methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside as shown in Table XXVIII. Only those compounds with relative retention volumes of 0.68, 0.73, 0.88, 1.00, 1.33 and 2.66 were present in significant amounts. The molar ratio of the pentamethyl glucosides to pentamethyl fructosides was found to be 0.69:0.31, as determined by their area ratios. Furthermore, the molar ratio of methyl 2,3,4-tri-O-methyl-β-D-glucopyranoside to that of the compounds with relative retention volumes of 1.33 and 2.66 (see Table XXVIII) was 0.34:0.66. The methanolysis products of octa-O-methyl sucrose are also shown in Table XXVIII.

TABLE XXVIII

Retention volumes of the products of methanolysis of hepta-O-methyl and octa-O-methyl sucrose relative to methyl 2,3,4,6-tetra-O-methyl α-D-glucopyranoside

Compounds	Standards	Octa-O-methyl sucrose	Hepta-O-methyl sucrose
methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside	0.68	0.68	0.68
" 1,3,4,6- " -O- " -β(?)-D-fructofuranoside		0.73	0.73
" " " " -α(?)-D- "		0.88	0.88
" 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside	1.00	1.00	1.00
" 1,3,4-tri-O-methyl-β(?)-D-fructofuranoside(1)			1.33
" " -α(?) " " (1)			1.49
" 2,3,4-tri-O-methyl-β-D-glucopyranoside	1.81		1.81
" 1,3,4-tri-O-methyl-β(?)-D-fructopyranoside(1)			1.91
" 2,3,4-tri-O-methyl-α-D-glucopyranoside	2.38		2.38
" 1,3,4-tri-O-methyl-α(?)-D-fructopyranoside(1)			2.66

1. These compounds were provisionally assigned these structures after the structure of hepta-O-methyl sucrose had been elucidated (see page 158).

DISCUSSION

The Snell Laboratories in 1954 developed a process for the preparation of sucrose esters using the transesterification reaction. The procedure was based on the reaction of sucrose with the methyl ester of a fatty acid in dimethylformamide solution using solid potassium carbonate as catalyst. The methanol formed in the reaction was removed *in vacuo* under conditions for fractional distillation (1). This process attracted considerable interest from industry as a means for the preparation of sucrose monoesters of the higher fatty acids for use as low cost, non-ionic detergents (5, 6).

The problem came into our laboratory six years ago; initially for the elucidation of the structure of sucrose monoester. However, when an attempt was made to prepare samples of the monoester for structural analysis, it soon became apparent that the product did not warrant such an investigation since it could not be prepared under fully controlled and reproducible conditions (3). For unknown reasons, highly variable yields were obtained and the products differed considerably in the degree of substitution, solubility and color even when the reaction was performed under apparently identical experimental conditions (3). Although other workers have claimed more success in this respect, a critical assessment of their

results clearly indicates that they have been equally unsuccessful in preparing a reproducible product in high yield (1,2,4,36). This lack of agreement, in their results and conclusions, was undoubtedly related to their empirical approach to the problem. It was considered, therefore, that this problem could only be solved by a systematic physical chemical investigation of the process for the preparation of the sucrose esters.

One of the requirements for such an investigation was the establishment of routine analytical methods of sufficient precision for the determination of the rates and extents of the reaction, and for the determination of the amounts of sucrose mono- and diester in the product. The development of these methods was difficult because of the experimental requirements of the transesterification reaction. All of the rate measurements, for example, had to be determined for a reaction which was carried out in a hygroscopic and high boiling solvent (dimethylformamide), at elevated temperatures and under reduced pressure, while one of the products of the reaction (methanol) was being removed continuously from the reaction. There is no previous record in the literature of a kinetic study having been made on such a system.

Analytical methods

The rates of the transesterification reactions were determined by following the decrease in concentration of pure methyl esters of the higher fatty acids using gas-liquid partition chromatography. All analyses were carried out on a four foot chromatographic column packed with a 10 percent W/W Apiezon M/Celite mixture, at 200°, using argon as the mobile phase. It was established that the concentration of methyl ester could be determined by this method to within $\pm$ 2 percent, from 10 to 100 percent of the range of concentrations encountered, when n-octadecane was used as an interral standard (see Table II and Figure II). Methyl myristate was used for most of the kinetic studies since it had been used in this laboratory for previous investigations (3), and had a convenient retention volume relative to n-octadecane (see Table I). Although Lovelock (67) had shown that the response of the ionization detector was not the same for hydrocarbons and methyl esters of fatty acids, due to the small difference in their ionization potentials, the methyl esters of myristic, palmitic and stearic acids gave the same molar response as n-octadecane under our experimental conditions.

At the end of the reaction period 25 ml aliquots of the reaction solution were taken to determine the

sucrose ester content. The dimethylformamide solution was first cooled to 20°, by the addition of solid carbon dioxide, and then extracted with a petroleum ether/ether mixture to remove the methyl ester of the fatty acid and n-octadecane. After removing the dimethylformamide, under reduced pressure at 70°, the residue was partitioned between n-butanol and 5 percent sodium chloride solution. The sucrose ester was extracted quantitatively into the n-butanol phase, and the catalyst and any unreacted sucrose was removed in the aqueous phase. Any sodium chloride in the n-butanol phase was precipitated by the addition of an equal volume of chloroform, and the resulting chloroform/n-butanol solution was dried with anhydrous sodium sulphate. After the solid materials were removed by filtration, the clear filtrate was diluted with a further aliquot of chloroform. It was found that only sucrose ester was present in solution if no precipitate or cloudiness was observed at this stage. The chloroform/n-butanol solution was taken to dryness under reduced pressure and the residue of sucrose ester dried over phosphorous pentoxide. A series of routine tests for the presence of impurities such as methyl myristate, n-octadecane, sucrose, sodium chloride and soap were then carried out on the sucrose ester (see page 40).

An obvious weakness, in the isolation procedure, was the presence of the catalyst in the reaction mixture during the period in which the dimethylformamide was being removed. Since the solution properties would change continuously as the solvent was being removed, it was possible that the composition of the sucrose ester which was isolated was not necessarily the same as that present in the original reaction mixture. It was realized that the neutralization of the catalyst, with an equivalent amount of acid, was a possible solution to the problem. However, in the initial stages of this work little was known about the extent to which the catalyst was used up in side reactions. There was moreover the possibility that a small amount of acid might be present during the removal of dimethylformamide if an attempt was made to neutralize the catalyst. The presence of acid in the reaction mixture would cause inversion of the sucrose and sucrose ester, which would be less desirable than any change in the product composition. The simple routine isolation procedure described above was adopted since experience showed that it gave high, reproducible yields of sucrose ester for reactions carried out under identical experimental conditions (e.g. see Table X). This procedure is only recommended for small volumes of the reaction solution which can be taken to dryness rapidly.

When an attempt is made to produce sucrose ester on a preparative scale, large volumes of solvent have to be removed and there is little doubt that the yield of sucrose monoester is reduced (see pages 80 and 108). It has been found that the addition of an equivalent amount of acetic acid or potassium hydrogen phthalate neutralizes the catalyst without causing inversion of the sucrose or sucrose ester (see pages 107 and 84).

Of the other analytical methods used, one worthy of mention is the separation of sucrose monoesters from sucrose and higher esters by chromatography on silicic acid using graded elution with methanol/chloroform solutions. The higher esters of sucrose were eluted with 5 percent methanol in chloroform and sucrose monoester with 10 percent methanol in chloroform. The recoveries were quantitative.

The transesterification reaction

To minimize side reactions, all of the reagents for the transesterification reaction were purified. The dimethylformamide was dried thoroughly by distillation from phosphorous pentoxide and only methyl esters which contained no free fatty acid were used. Potassium carbonate was dried at 180° for twenty-four hours and then stored *in vacuo* over phosphorous pentoxide. Methanolic

solutions of the catalyst were prepared with methanol which had been distilled from magnesium. The sucrose was stored *in vacuo* over phosphorous pentoxide. Furthermore, considerable care was taken to remove and exclude moisture from the reaction solution. All parts of the glass reaction apparatus were dried at 180° overnight and then allowed to come to room temperature, over phosphorous pentoxide, in a dessicator. The standard reaction solution was 18.75, 6.25 and 1.25 x 10^{-2} molar in sucrose, methyl ester, and catalyst respectively. Since sucrose contains water of crystallization, the former was dissolved, with mechanical stirring, in the dimethylformamide and the resulting solution was conditioned at 80° and 75 mm pressure for 30 minutes in order to remove all traces of water. The methyl ester and n-octadecane was then added, as a solution in petroleum ether, to the dimethylformamide sucrose solution. After removing the petroleum ether under reduced pressure, the reaction solution was conditioned for a further 30 minutes before the catalyst was added to start the reaction. During the entire procedure a stream of dry nitrogen was passed through the dimethylformamide solution. As a further precaution the air bleed, which was used to control the vacuum in the reaction apparatus, was placed between two vapour traps maintained at -70°.

It was realized, moreover, that different specimens of the individual reagents would be used during the investigation, and that this could affect the reproducibility of the results. This would apply particularly to the dimethylformamide which was being used as a solvent for the reaction. This difficulty was partially overcome by using identical specimens of the reagents for all experiments dealing with one reaction variable. In this way it was possible, for example, to compare the rates of reaction, even if they were not the absolute reaction rates, within a selected group of experiments. It was found that reproducible rates and extents of reaction were obtained when this experimental procedure was used under conditions for homogeneous catalysis (see below).

Kinetic studies

Equipped with these experimental procedures and analytical tools, a kinetic investigation of the reaction was undertaken. It seemed desirable to begin with an examination of the procedure, recommended by the Snell Laboratories (1, 2), which used solid potassium carbonate as the catalyst for the reaction. It soon became apparent that reactions performed at 80° under reflux, and initiated by solid potassium carbonate, proceeded at unpredictable

rates and to variable extents (see Tables III and IV, and Figure III). Furthermore the yields of sucrose ester varied from 40 to 87 percent, and the reaction times from 4 to 24 hours (see Tables III and IV). Also, although all of the potassium carbonate did not dissolve in any of our experiments, it was apparent that variable amounts went into solution. It became evident that the variation in the rates of reaction and yields of product was to an important degree related to the rate of dissolution of the catalyst. The rate of reaction was substantially increased (almost two-fold) by using finely divided potassium carbonate distributed over Celite, and were adversely affected by the presence of water in solution (see Tables V and VI, and Figure IV). This is in contrast to results of other workers (3, 4, 36) who found that solid potassium carbonate dissolved completely in the reaction solution. Furthermore, experiments 11 and 12 using homogeneous solutions of potassium carbonate in dimethylformamide and dimethylformamide/sucrose, respectively, indicated that the reaction did not proceed by heterogeneous catalysis (see Table VII and VIII). The former solution was only 0.0014N in potassium ion. The precise nature of these effects were not established since it was by now clear that a satisfactory solution to the problem would require the transesterification reaction to be carried out under

conditions for homogeneous catalysis.

It was therefore decided to add the potassium carbonate as a solution in methanol. At this point a surprising (to us) observation was made. When a saturated methanolic solution of potassium carbonate was prepared, under reflux, the titration of the solution revealed the presence of strong base. Furthermore, the amount of strong base increased the longer the solution was heated. It was apparent, therefore, that heating led to the loss of acid through the evolution of carbon dioxide. By analogy the reaction of sucrose with potassium carbonate should lead to the formation of potassium sucrate in high concentration. The formation of sucrose myristate using solid sodium hydride as the catalyst suggested, moreover, that sucrate ion was the reactive species which catalysed the reaction (see experiment 14, Tables VII and VIII). Consequently potassium methoxide should catalyse the reaction as efficiently as potassium carbonate.

The standard transesterification procedure was therefore modified to enable methanolic solutions of the catalyst to be added to the reaction solution. After the dimethylformamide solution of sucrose, methyl ester, and n-octadecane had been conditioned (see above), methanolic solutions of base were injected with a hypodermic syringe into the reaction solution through a silicone rubber seal.

This was accomplished with the apparatus still under reduced pressure. The reaction solution at this stage had a milky appearance due to the presence of a fine precipitate of potassium sucrate. The latter redissolved rapidly as the methanol was being removed by distillation, to leave a homogeneous solution which was 0.0125N in potassium ion. It was found that the reaction now proceeded at reproducible rates and was essentially independent of whether potassium carbonate or potassium methoxide was used as a source of catalyst. Approximately 92 percent of the methyl myristate had reacted in only 2-1/2 hours, with the formation of sucrose ester in approximately 82 percent yield (see Tables IX and X). Infrared analysis also showed that the products of reaction were identical. Solutions of potassium hydroxide in methanol (0.0125N in potassium ion) could also be used to prepare reaction solutions of substantially identical properties as those obtained from methanolic solutions of either potassium carbonate or potassium methoxide. In this case 86 percent of the methyl myristate had reacted in 2-1/2 hours and gave sucrose myristate in 74 percent yield (see Tables IX and X). The rates of reaction which were obtained using conditions for homogeneous catalysis were much superior to those obtained by other workers (1, 2, 3, 36, 38).

It was also demonstrated that saturated aqueous solutions of potassium carbonate and potassium hydroxide catalysed the reaction, but led to the formation of detectable amounts of soap. In these experiments approximately 78 percent of the methyl myristate had reacted in 2-1/2 hours, to give sucrose myristate in 70 percent yield (see Tables XI and XII). A similar result was obtained using a saturated methanolic solution of potassium hydroxide as catalyst. Superior initial rates of reaction were obtained when a saturated solution of potassium methoxide was used as catalyst. In this case 58 and 95 percent reaction had occurred in 30 minutes and 2-1/2 hours, respectively, to give sucrose myristate in 82 percent yield (see Tables XI and XII). This compares favourably with 43 and 95 percent reaction, in the same time intervals, using dilute methanolic solutions of the catalyst (see Table XVII). The discrepancy in the initial rates of reaction was undoubtedly related to the efficiency with which the methanol was being removed from the reaction solution. Although only 0.5 ml of methanol (20 mM) can be formed, theoretically, from the methyl myristate during the transesterification reaction, the catalyst was added to the reaction solution in 5 ml of methanol. Any slight differences in the reaction conditions will therefore be more critical in the initial stages of the reaction. This

is consistent with the observation of Komori and his associates (38), that the rate of reaction of sucrose with a methyl ester in formylmorpholine, at constant temperature, increased when the pressure in the system was decreased. This situation could only arise if the methanol was being removed more efficiently at the lower pressures. With our apparatus, however, it was not possible to operate at pressures lower than 75 mm at 80°, because the dimethylformamide distilled over and thereby altered the concentrations of the reactants. Furthermore, in order to get reproducible results, an attempt was made to use the same experimental conditions throughout the investigation. For example, apart from using the same reaction temperature and pressure etc. the solvent for the reaction (dimethylformamide) was refluxed to a point, in the Vigreaux column, two inches from the neck of the reaction flask. However, dry nitrogen was bubbled through the reaction solution to facilitate the removal of methanol. Any small variation in the experimental conditions, however, could affect the rate at which methanol was being removed and this in turn would influence the rate of reaction. It was found, moreover, that although reproducible aliquots of a saturated solution of potassium methoxide could be delivered, it was not possible, due to the viscous nature of the solution, to routinely deliver

aliquots of the solution containing definite known concentrations of catalyst. Most of our work has consequently been carried out using dilute methanolic solutions of the catalyst, which were much easier to handle.

It was realized that the initial rates of reaction might be improved by adding the methanolic solution of the catalyst to the reaction solution before the methyl myristate/n-octadecane mixture. This would enable the methanol to be removed during the conditioning period (see above) and before the transesterification reaction was initiated. It was found, however, that a solution of the methyl myristate/n-octadecane mixture in a solvent, such as petroleum ether, was the only practical method of adding accurate amounts of these reagents to the reaction solution. The addition of petroleum ether to reaction solutions containing sucrose and potassium sucrate caused the latter to precipitate. This precipitate had a tendency to coagulate and did not redissolve rapidly. The removal of the petroleum ether from the reaction solution, moreover, caused small particles of the potassium sucrate to be entrained in the distillate, and these particles then adhered to the walls of the reaction vessel. Furthermore, the evaporation of the petroleum ether caused the reaction solution temperature to fall below 70°, and it took at

least 30 minutes to return to the reaction temperature 80°. All of these factors made it impossible to reproduce the catalyst concentration and rate of reaction, so this procedure was not adopted.

The effectiveness of the procedure for removing the methanol from the reaction solution was tested as follows. At intervals, during the course of the transesterification reaction, samples were removed and placed in sealed glass tubes. The latter were then kept at the reaction temperature to achieve equilibrium. Also, the methyl myristate content of the reaction mixtures, at the various times of sample withdrawal, were determined (see Table XXIII). Assuming that the amount of methanol at equilibrium was derived entirely from the methanol liberated after the sample was withdrawn, then its concentration could be calculated from the methyl myristate content. Equilibrium constants for the reaction,

Sucrose + Methyl myristate $\rightleftharpoons$ Sucrose myristates + Methanol,

were then calculated from this data. It can be seen from Table XXIV that if it was assumed that only sucrose monomyristate was formed the equilibrium constant K_3 varied between the limits 0.2 and 0.36 with an average value of 0.26, whereas if a correction was made for the presence of sucrose dimyristate the new values, denoted K_2, varied

between the limits 0.17 to 0.32 with an average value of 0.23. It can be concluded, therefore, that the methanol is being removed rapidly from the reaction solution since the accumulation of methanol would result in a much wider range of values for K_2 and K_3. The latter, moreover, would have the highest values in the initial stages of the reaction, and this was not observed.

Experiments directed at obtaining an accurate measure of the equilibrium constant K_2 (see above) were carried out by approaching the equilibrium condition from both sides. Dimethylformamide reaction solutions which were 18.75, 6.25, 6.25 and 0.7×10^{-2} molar in sucrose, methyl myristate, methanol and potassium methoxide, respectively, in one experiment, and 6.25, 6.25 and 0.7×10^{-2} molar in sucrose monomyristate, methanol and potassium methoxide, respectively, in another experiment, were allowed to equilibrate at 80°. The rate of equilibration was followed by gas-liquid partition chromatography using n-octadecane as an internal standard (see Table XXV and Figure XI). From a knowledge of the initial concentrations of the reactants and the concentration of methyl myristate at equilibrium, the concentrations of the compounds at equilibrium could be determined (see pages 98 and 101). From these concentrations the equilibrium constant K_2 was found to have a value of 0.57 in both cases. The close

agreement in these results is probably fortuitous, although it is not considered likely that the actual value of K_2 will differ significantly from that found experimentally. This means of course, that the formation of methyl myristate is favoured in the equilibrium reaction, and is consistent with the results obtained by Fehland and Adkins for the equilibrium reactions of a series of aromatic and aliphatic alcohols with methyl acetate. These workers showed, that in the presence of aluminum alkoxide, the equilibrium reactions always favoured the formation of methyl acetate. For example, the equilibrium constant had a value of 0.71 when dodecyl alcohol was used. They also found that the value of the equilibrium constant decreased when branching occurred in the alcohol (68). Juvet and Wachi have also shown that the methanolysis of simple aliphatic esters, in the presence of an acid catalyst, under equilibrating conditions favours the formation of methyl esters (54). It is realized, of course, that comparing results obtained from quite different reaction mixtures has to be made with caution. However, the value of K_2 establishes the necessity for removing methanol efficiently in the transesterification reaction.

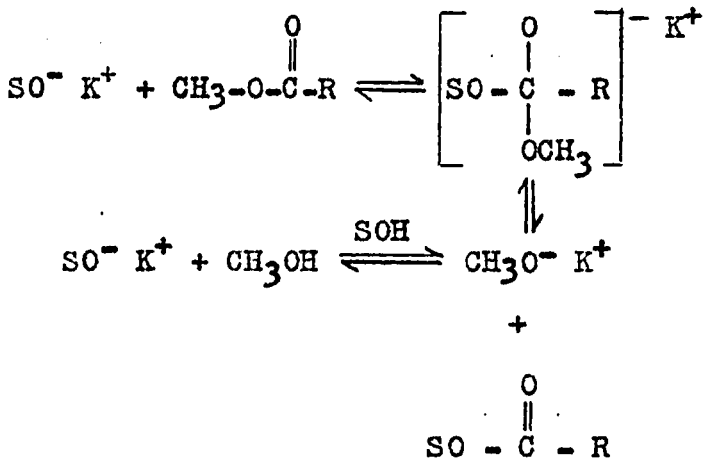
It became obvious, early in the investigation, that the transesterification reaction followed pseudo first order kinetics in the methyl ester of the fatty acid (see

Tables XVIII and XIX). Evidence that sucrate ion was the reactive species which catalysed the reaction was provided when the extent and rate of a reaction, using a methanolic solution of potassium carbonate as catalyst, was strongly depressed when a stream of carbon dioxide was passed through the solution instead of the usual nitrogen (see Figure V). Only 22 percent of the methyl myristate had reacted in 2-1/2 hours to yield 19 percent of sucrose ester. When the carbon dioxide was replaced by nitrogen, the reaction proceeded as usual and 96 percent of the methyl myristate had reacted by the end of a further 2 hours reaction time, and gave sucrose ester in 84 percent yield (see Tables XVI and XVII). This requires that sucrose (SOH) and potassium carbonate react according to the following scheme,

$$SOH + K_2CO_3 \rightleftharpoons K.OS + KHCO_3$$
$$SOH + KHCO_3 \rightleftharpoons K.OS + H_2CO_3$$
$$H_2CO_3 \longrightarrow CO_2\uparrow + H_2O\uparrow$$

to form potassium sucrate (K.OS). Furthermore the fact that equivalent amounts of potassium carbonate, potassium methoxide, sodium methoxide and lithium methoxide gave identical rates of reaction can only be satisfactorily explained if sucrate ion is the effective catalyst for the reaction (see Table XV).

The transesterification reaction must therefore be a bimolecular reaction, and the mechanism of transesterification must involve nucleophilic attack of the methyl ester by the sucrate ion,



In view of this mechanism, the rate of the transesterification reaction should be dependent only on the concentrations of sucrate ion and methyl ester. However, the actual rate of reaction would also depend on the rate of reformation of the methyl ester in the reverse direction, and this will depend on the efficiency with which the methanol is removed from the reaction solution. Since the methanol is kept at an insignificantly low level under our experimental conditions (see above), the sucrate ion concentration should remain at virtually constant level throughout the course of the reaction. Thus, the rate of reaction should be directly proportional to the sucrate ion concentration. It can be seen from Table XIX that this is

the case for reaction solutions which were up to 3.125×10^{-3} molar in potassium sucrate (one milliequivalent of potassium sucrate per 320 ml of reaction solution). For example the pseudo first order rate constants were 0.75 and 1.43×10^{-4} sec^{-1} for reaction solutions which were 1.5625 and 3.125×10^{-3} molar in potassium sucrate, respectively. At higher concentrations of potassium sucrate the reactions behave in an non-ideal manner (see Figure VI). The pseudo first order rate constants were only 2.36, 3.33 and 3.67 $\times 10^{-4}$ sec^{-1} for reaction solutions which were 6.25, 12.5 and 25×10^{-3} molar (2,4 and 8 milliequivalents of potassium sucrate per 320 ml of solution, respectively) in potassium sucrate (see Table XIX). Consequently an equilibrium must exist between a reactive (dissociated) and unreactive potassium sucrate in the reaction solution. It would require a separate investigation to determine the exact nature of the unreactive potassium sucrate. Nevertheless, it is probable that the latter is present as undissociate potassium sucrate or as some kind of close ion pair. The concentration of reactive sucrate ion, for any concentration of potassium sucrate up to 25×10^{-3} moles $liter^{-1}$, can be determined from the graph shown in Figure VI. The concentration of sucrate ion corresponding to 25×10^{-3} moles $liter^{-1}$ of potassium sucrate, for example, is only 7.5×10^{-3} moles $liter^{-1}$ (2.4 milliequivalents in

320 ml of reaction solution). It is clear therefore, that the transesterification reaction is a second order reaction which is first order in methyl ester and sucrate ion, respectively. This is consistent with the results of Taft and his coworkers who established that the methanolysis of l-menthyl benzoate, in the presence of sodium methoxide, was a bimolecular reaction showing first order kinetics in ester and alkoxide ion, respectively (46). Any claim by Herstein and his coworkers (4, 36) that the reactive species which catalyzes the reaction is some kind of chelate complex of sucrose and a metallic ion is clearly disproved by this work. Indeed, the fact that these workers could arrive at this conclusion suggests that they are unaware of the mechanisms of ester-ester interchange, hydrolysis and transesterification reactions.

In view of the above considerations, the rate of reaction should be independent of the concentration of sucrose as long as the methanol is being removed effectively. This was found to be the case, and no appreciable rate difference was noted by doubling or halfing the initial sucrose concentration. The methyl myristate had reacted to the extent of 96 percent with a yield of sucrose ester of 82-85 percent when 18.75 and 12.5 x 10^{-2} moles $liter^{-1}$ of sucrose were used in the reaction. When the concentration of sucrose was reduced to 6.25 x 10^{-2} moles $liter^{-1}$, 85

percent of the methyl myristate reacted to give sucrose myristate in approximately 70 percent yield (see Tables XIII and XIV). In the latter case, a precipitate of potassium sucrate was present during the entire reaction period. In view of the high rate of reaction, however, it is felt that if all of the catalyst had been in solution the rates of reaction at the three concentrations of sucrose would have been identical.

The nature of the cation has little effect on the rate of the reaction. Experiments using equivalent amounts of potassium carbonate, lithium methoxide, sodium methoxide and potassium methoxide, as a potential source of sucrate ion, gave essentially the same rates of reaction (see Table XV). It can therefore be predicted with confidence that caesium and rubidium methoxides would behave similarly. The only possible gain that could be obtained by changing the cation would be in the solubility of the metal sucrate in the reaction solution. It was observed that lithium sucrate was the only catalyst which did not give an initial precipitate when it was added to the reaction mixture. The saturation levels of the metal sucrates, other than potassium sucrate, have not been determined. These results are in contrast to cation-sucrose complex theory of Herstein which must necessarily predict different rates of reaction for the different cations (4, 36).

The chain length of the fatty acid also has little effect on the rate of the reaction. Pseudo first order rate constants for methyl myristate, methyl palmitate and methyl stearate, were 3.33, 3.17 and 2.96 x 10^{-4} sec^{-1} respectively when the sucrose, methyl ester and potassium sucrate concentrations were 18.75, 6.25 and 1.25 x 10^{-2} moles $liter^{-1}$ of reaction solution, respectively (see Table XVIII).

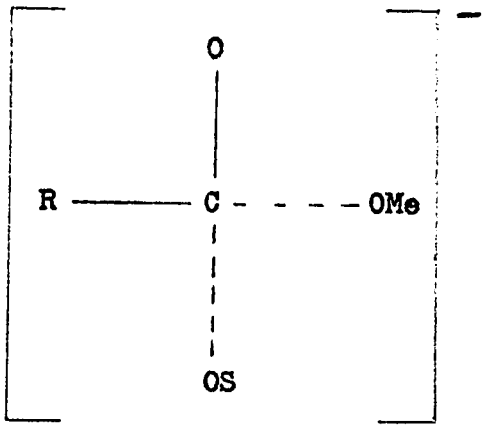
The temperature coefficient for the reaction was determined by carrying out the standard transesterification reaction at two temperatures (80° and 97°) and two concentrations of potassium sucrate (6.25 and 12.5 x 10^{-3} moles $liter^{-1}$). At the lower catalyst concentration the pseudo first order rate constants for the reaction were 2.36 and 4.51 x 10^{-4} sec^{-1} at 80° and 97°, respectively. When the catalyst concentration was doubled the pseudo first order rate constants were found to be 3.33 and 5.52 x 10^{-4} sec^{-1} at the two temperatures, respectively (see Table XX). It was observed that the potassium sucrate did not dissolve as rapidly as usual for the reaction using 12.5 x 10^{-3} moles $liter^{-1}$ of potassium sucrate at 97°. A duplicate experiment at this temperature exhibited the same behaviour and gave an identical rate of reaction. Although the solubility characteristics of potassium sucrate at 97° was not studied, the above results suggest that the latter has

a negative solubility temperature coefficient. This could explain why the rate of reaction doubles at the lower catalyst concentration (see Figure VII), but only increases by a factor of 1.6 at the higher concentration of catalyst for a 17° increase in the temperature of the reaction. Since the potassium sucrate appeared to behave normally at the 6.25×10^{-3} moles liter^{-1} level, at both reaction temperatures, the corresponding rate constants were used to calculate the activation energy (9.9 kcal mole^{-1}) and the frequency factor (0.7×10^{5} liters moles^{-1} sec^{-1}) for the reaction. The thermodynamic constants for the reaction were then calculated and found to be,

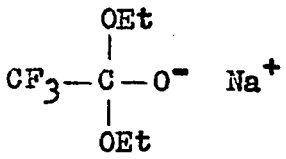
$$\Delta F^{\ddagger} = 22 \text{ kcal mole}^{-1},$$
$$\Delta H^{\ddagger} = 9 \text{ kcal mole}^{-1},$$
$$\Delta S^{\ddagger} = -38 \text{ entropy units.}$$

The low frequency factor and large negative entropy is usually associated with reactions in which there is electrorestriction of the solvent molecules due to the formation of a highly polar activated complex (69, 70). Reactions which exhibit this behaviour also have negative volumes of activation, and the rates of reaction are accelerated by pressure (70). These phenomena are observed in the acid and base hydrolysis of esters and amides (71, 72, 73), and in the transesterification of esters with an alcohol using

base catalysis (45, 46). The activated complex for the reaction between sucrate ion and the methyl ester of a fatty acid can be represented as



This asymmetric complex is consistent with the tetrahedral intermediate which is formed in the hydrolysis of esters by base (53, 74), and with the addition compound



which is formed by the reaction of ethyl trifluoroacetate with sodium ethoxide (75, 76). The low activation energy (9.9 kcal mole^{-1}) for the transesterification reaction is similar to the values found for the hydrolysis of a series of isomeric octyl acetates with base (77). Since the transesterification reaction has a low activation energy and is highly endothermic it can be expected that the

activated complex will resemble the products of reaction more than the reactants (78).

Equilibrium studies

Before an investigation could be made of the equilibrium existing between sucrose myristates with differing degrees of substitution, pure samples of the latter had to be isolated. Sucrose mono- and dimyristate were isolated from the combined reaction products of three transesterification reactions using 60, 20 and 4 mM of sucrose, methyl myristate and potassium sucrate, respectively. The sucrose myristates were obtained in 77 percent yield, based on the complete conversion of all of the methyl myristate to sucrose monomyristate, and 93.5% of the original myristoyl group content of the reaction mixtures was accounted for. The remainder of the methyl ester probably formed soap during the isolation procedure. Chromatography of the sucrose myristates on silicic acid using graded elution with methanol/chloroform solutions gave sucrose mono- and dimyristate in 53 and 24 percent yield respectively (see Table XXI). This corresponds to a molar ratio of sucrose mono- to dimyristate of approximately 3:1. Precipitation from acetone gave pure sucrose monomyristate, as a white amorphous powder, in 47 percent yield. Similarly, sucrose dimyristate, in a high state of purity, was obtained in approximately 18 percent yield as

a white amorphous powder.

Using a chromatographically pure sample of sucrose monomyristate it was possible to establish that the equilibrium constant K_1 for the reaction,

Sucrose + Sucrose dimyristate $\rightleftharpoons$ 2 Sucrose monomyristate,

to be 1.9 ± 0.1. These results were obtained by equilibrating sucrose monomyristate and sucrose in dimethylformamide and in the presence of potassium sucrate. Although the equilibrium was not approached from both sides, the values for K_1 were obtained at two different sucrose concentrations. Both reaction solutions were 6.7 and 1.33×10^{-2} molar in sucrose monomyristate and potassium sucrate, and contained 6.7 and 3.23 moles of sucrose per liter of solution, respectively. The lower value for K_1 was obtained at the lowest sucrose concentration. This is reasonable since the error due to the presence of sucrose myristates with more than two myristoyl groups per molecule of sucrose would be higher in this case. Since no significant amounts of these higher esters of sucrose were detected at the higher concentration of sucrose, the corresponding value for K_1, namely 2, was used for all calculations in which this equilibrium constant was required.

The theoretical yields of sucrose monomyristate corresponding to any initial concentration of sucrose and

methyl myristate could be determined from a knowledge of K_1 (see page 103), provided it was assumed that there was quantitative conversion of the methyl myristate to sucrose mono- and dimyristates. From Figure XII, for example, it can be seen that 0.74, 0.80 and 0.83 moles of sucrose monomyristate are formed when 3, 4 and 5 moles of sucrose are used per mole of methyl myristate in the transesterification reaction. This corresponds to a 92, 93.7 and 95 percent yield of sucrose myristates (sucrose mono- and dimyristates), respectively, at the three sucrose concentrations. Thus it is clear that little is gained by the use of a sucrose to methyl ester ratio of greater than 3. In all of our experiments the methyl myristate had reacted to the extent of approximately 92 percent and gave a yield of sucrose myristates of approximately 83 percent by weight. From equation 11 it can be calculated that 92 percent reaction would give 0.80 and 0.06 moles of sucrose mono- and dimyristates, respectively, corresponding to a theoretical weight yield of sucrose myristates of 87 percent, and a yield based on the amount of methyl myristate which had reacted of 94 percent. However, since most of our yields were obtained on small aliquots of the reaction solution a simple procedure was now developed as a result of our kinetic studies to make sucrose myristates on a preparative scale.

The concentrations of sucrose and methyl myristate in dimethylformamide were 18.75 and 6.25 x 10^{-2} molar, respectively. After conditioning these reagents in the usual manner at 80° and 65 mm pressure for 15 minutes, sodium methoxide was added to the solution. The reaction solution was 1.25 x 10^{-2} molar in sodium sucrate. When the reaction mixture had been conditioned for a further 15 minutes the methyl myristate was added to the reaction solution by means of a hypodermic syringe. The reaction was continued for 150 minutes during which time 50 ml of dimethylformamide had distilled over. At the end of this period the reaction was stopped by the addition of excess glacial acetic acid. After working up the product the sucrose myristate was obtained in 84 percent yield (see page108). This result is in excellent agreement with the predicted yield of 87 percent (see above), and with the yields which we have obtained during our investigation (see Tables X and XIV).

It was also established, using uniformly C^{14}-labelled sucrose, that the rate of the exchange reactions,

Sucrose* + Sucrose dimyristate $\rightleftharpoons$ Sucrose myristates*

and

Sucrose* + Sucrose monomyristate $\rightleftharpoons$ Sucrose myristates*,

was too rapid to expect any product control through kinetic control. In other words, at any time during the transesterification reaction, the product prevailing is the thermodynamic product under the conditions which prevail. The reaction solutions were approximately 10, 10 and 2 x 10^{-2} molar in C^{14}-labelled sucrose, sucrose myristate (mono- or dimyristate) and potassium sucrate, respectively. Samples were taken at intervals over a period of 65 minutes and the sucrose myristates isolated by chromatography. The specific activity of the sucrose myristates was then determined. From Table XXII it can be seen that approximately 65-80 percent of the theoretical activity was present in the isolated sucrose myristates after only 10 minutes reaction. The discrepancy between the specific activity of the sucrose ester isolated after 65 minutes and the theoretical value, is undoubtedly due to the presence of poly-substituted esters of sucrose. It is clear, from these results, that the sucrose esters formed in the transesterification reaction will have a higher sucrose monoester content in the initial stages of the reaction than at the completion of the reaction. We have not tested this prediction but make it with confidence. This is in direct contrast to the claims of other workers (1, 2, 3, 4, 36) that the reaction proceeds by way of the rapid formation of sucrose diester. Furthermore any claims that

water (2) and the catalyst concentration influence the product composition (4, 36) are not justified, and the optimal conditions for producing sucrose monoester evaluated by orthogonal statistical design in the manner suggested by Herstein and coworkers (4, 36), is clearly not valid.

It is of interest that the hydrolysis of esters and amides in acidic or basic media is greatly accelerated when a suitable neighbouring group participates. Bender has shown that phthalamic acid was hydrolyzed 10^5 times faster than benzamide, and has attributed the enhanced rate of reaction to the direct intramolecular participation of the neighbouring carboxyl group (79). A similar effect has been reported by Bartlett and Greene in the hydrolysis of methyl 2-hydroxytriptoate by base (80). Henbest and Lovell have also shown that hydroxyl group participation was responsible for the enhanced rates of hydrolysis of those isomers of 3-acetoxy-5-hydroxy cholestane and coprostane which had an axial acetyl group (81). Furthermore, Kupchan and Johnson have established that the abnormally high rate of hydrolysis of the C_{16} acetate ester of cevine was due to the participation of the C_{20} hydroxyl group (82), and have shown that these groups were cis-1,3-diaxially oriented with respect to one another. This type of participation was also responsible for the facile

migration of an acetyl group from the 4- to the 6-position of sucrose (22). It is possible, therefore, that hydroxyl group participation may be partly responsible for the rapid exchange of C^{14}-labelled sucrose with sucrose monomyristate, and may even assist in the formation of sucrose monoesters. For the same reason any ester groups on the secondary hydroxyl groups of sucrose would be expected to migrate rapidly to the 6-, 6'- and 1'-positions of sucrose, respectively. Consequently any sucrose monoester formed in the transesterification reaction should have most of the ester groups at the 6-, 6'- and 1'-positions. It has been shown experimentally that 6- and 6'-myristoyl sucroses are formed exclusively in the transesterification reaction (see below).

Side reactions in the transesterification reaction

Since other workers (2, 3, 4) had reported that side reactions occurred in the transesterification reaction, an attempt was made to establish the validity of these observations. A standard reaction mixture of sucrose and methyl myristate, in the absence of catalyst, was maintained at 80° and 75 mm pressure for 6 hours. Periodic analysis of the reaction solution by gas-liquid partition chromatography revealed that the methyl myristate concentration remained constant, as might be expected, over

the reaction period. This is in contrast to the claim of Herstein and his coworkers (4, 62) that sucrose can react with a methyl ester in dimethylformamide solution to form free fatty acids (4, 36). Although it is difficult to understand how these workers could suggest such a reaction, there is a possible explanation for the presence of free fatty acids in their products. If their reaction mixtures contained moisture, hydrolysis of the esters in solution by base could result in the formation of salts of the formula: $C_nH_{2n+1}COOK.C_nH_{2n+1}COOH$ (83), which would give free fatty acid and normal soaps when the product is isolated. It was shown, moreover, that no side reactions occurred when methyl myristate was heated in anhydrous dimethylformamide using potassium methoxide as catalyst. This disproves any claim (36) that N,N-dimethylmyristamide is formed in the reaction. Furthermore the fact that the rate constants for the transesterification reaction remain virtually constant over the reaction period (see Tables XX, XIX and XVIII) would suggest that little or no catalyst is used up in side reactions. The absence of soap in our reaction products is consistent with this observation. This is in contrast to the results of other workers who report that the catalyst is used up during the reaction, and that soap is formed in significant amounts (1, 2, 3, 36). The reason for this apparent discrepancy in the experimental

results undoubtedly lies in the fact that anhydrous conditions prevailed during this investigation, whereas the reaction solutions of other workers contained moisture.

The structure of sucrose monomyristate

At this point, we felt it worthwhile to examine the structure of sucrose monomyristate. A chromatographically pure sample of the latter was methylated using silver oxide and methyl iodide in dimethylformamide solution (61, 62) using finely divided calcium sulphate as a drying agent. The crude yield of product was quantitative. However, chromatography on silicic acid showed the product to contain about 2 percent of the original myristoyl content as methyl myristate (see Table XXVI). Chromatographically pure hepta-O-methyl sucrose monomyristate, obtained in 92 percent yield was transesterified in methanol to yield hepta-O-methyl sucrose in 92 percent yield. The methoxyl content, 48.2 percent, of the purified hepta-O-methyl sucrose was in good agreement with the theoretical value of 49.3 percent. Hydrolysis with 0.5N sulfuric acid gave a product which on paper chromatography using butanol-ethanol-water 5:1:4 showed the presence of three components when sprayed with p-anisidine hydrochloride. Two of these spots (reddish brown) with R_{TG} values of 1.0 and 0.81 corresponded to

2,3,4,6-tetra-O-methyl glucose and 2,3,4-tri-O-methyl glucose. The third spot with an R_{TG} value of 0.89 was yellow (ketose) and agreed with the reported value for 1,3,4-tri-O-methyl fructose (66). When the paper was sprayed with resorcinal only two pink spots (ketose), with R_{TG} values of 1.00 and 0.89, were obtained. These values agree with the reported values for 1,3,4,6-tetra-O-methyl fructose and 1,3,4-tri-O-methyl fructose, respectively (66). Therefore it was evident that the hepta-O-methyl sucrose was almost entirely a mixture of only 2,3,4,1',3', 4',6'- and 2,3,4,6,1',3',4'-hepta-O-methyl sucroses.

Methanolysis of the mixture of hepta-O-methyl sucroses (3 percent methanolic hydrogen chloride for five hours) and gas-liquid partition chromatographic analysis of the methyl glycosides revealed the presence of ten compounds. The theoretical number of methyl glycosides which can be formed from the above mixture is ten (see Table XXVIII). The relative amounts of the fully methylated D-glucopyranosides and D-fructofuranosides was 0.69 to 0.31. Since experience has shown that the methyl furanosides of fully, or partially, methylated hexoses have smaller retention volumes then the corresponding methyl pyranosides (57, 105) when analyzed by gas-liquid partition chromatography, the compounds with relative retention volumes of 1.33 and 2.66 (see Table XXVIII) are probably

the methyl furanoside and pyranoside of 1,3,4-tri-O-methyl-?-D-fructose, respectively. The relative amounts of these compounds to that of methyl 2,3,4-tri-O-methyl-β-D-glucopyranoside was found to be 0.66 to 0.34 (see page 120).

The nuclear magnetic resonance spectrum for the mixture of hepta-O-methyl sucroses showed two signals (doublets) for the anomeric hydrogen, at low field, with a common spin-spin coupling constant of 3.5 cycles per second. The relative intensities of the two doublets was 0.65 to 0.35 and the doublet at lowest field had the greater intensity (see Figure XIII).

Reaction of the mixture of hepta-O-methyl sucroses with p-toluenesulphonyl chloride in pyridine produced hepta-O-methyl sucrose monotosylate in quantitative yield. The nuclear magnetic resonance spectrum again showed the presence of two doublets for the anomeric hydrogen with a relative intensity of 0.65 to 0.35 (see Figure XIV). When this monotosylate was treated with sodium iodide in acetone at 105° for 15 hours, hepta-O-methyl iodo-deoxy sucrose was obtained in 97 percent yield. The nuclear magnetic resonance spectrum of this compound revealed that 90 percent of the tosyloxy groups had been replaced by iodine (see page 116). When this compound was reduced with hydrogen and palladium on charcoal in

methanol, containing excess diethylamine, an iodine-free compound was obtained in 87 percent yield. The nuclear magnetic resonance spectrum of this compound showed two signals for C-methyl groups, in the form of doublets, with the characteristic spacing of 6.1 cycles per second for methyl groups substituted on a carbon carrying one hydrogen (64). The relative intensities of these signals were once again in the ratio of 0.65 to 0.35, and the signal at highest field had the greater intensity (see Figure XV). Therefore, the methyl groups must be at the 6'- and 6-positions of sucrose since these are the only two positions in which a methyl group could be attached to a carbon atom bearing a hydrogen. Furthermore, the gas-liquid partition chromatographic analysis of the products of methanolysis of hepta-O-methyl sucroses showed that more pentamethyl glucoside was formed then pentamethyl fructoside. This can only mean that the myristoyl content at the 6'-position is greater than at the 6-position of sucrose. It can be concluded, therefore, that sucrose monomyristate has about 0.62 of the myristoyl group at the 6'-position, 0.28 at the 6-position and 0.10 at the other positions. It is likely that most of the latter fraction is on the third primary hydroxyl, namely the 1'-position of sucrose. This is in direct contrast to the claims of other workers that sucrose monoester is substituted almost

exclusively at the 6-position (41) or the 1'-position (3) of sucrose.

The results obtained on the structure of sucrose monomyristate by first tosylating the ester and then reacting the tosylated product with sodium iodide in acetone were very misleading. A reaction sequence of this type revealed that 1.84 tosyloxy groups were replaced by iodine in sucrose monomyristate 5.18-tosylate. Since previous work in this laboratory had shown that only the 6- and 6'-positions in polysubstituted tosyl esters of sucrose were replaced by iodine (3, 30), the above result would require 90 percent of the myristoyl groups to be at the 1'-position of sucrose. This erroneous result is probably due to the replacement of secondary tosyloxy groups either by acyloxy participation or by the direct attack of iodide ion. It should be noted that tosyloxy groups at the 4- and 4'-positions of sucrose are suitably trans orientated with respect to an ester group on the 6- and 6'-positions, respectively. This type of replacement is to be expected in view of the conversion of the ditosyl esters of α- and β-monoglycerides of the higher fatty acids into allyl esters by the reaction of sodium iodide in acetone (85). This result could only be obtained if the carbonyl of the ester group participated in the replacement of the tosyloxy groups by iodine.

Since the composition and structures of the isomeric hepta-O-methyl sucroses have been established, it is now possible to assign the chemical shifts for their respective anomeric hydrogens. The signal (doublet) with a τ value of 4.53 must have been due to the presence of 2,3,4,6,1',3',4'-hepta-O-methyl sucrose since the intensity of the signal corresponds with the concentration of the compound in the mixture. By similar reasoning the signal with a τ value of 4.73 was due to 2,3,4,1',3',4',6'-hepta-O-methyl sucrose (see page 158). This would suggest that nuclear magnetic resonance could be used to obtain information on the structures of polysaccharides. For example, the isomeric hepta-O-methyl sucroses had a common spin-spin coupling constant of 3.5 cycles per second for the anomeric hydrogen. Since coupling constants are independent of the applied field its order of magnitude establishes that the hydrogens on the 1- and 2-positions of sucrose are gauche orientated with respect to one another (86). This can only be true if sucrose has an α-glucosidic linkage. Since the conformations of most of the common hexoses and pentoses are known, information on the way in which the monosaccharide units are linked in polysaccharides could possibly be obtained.

The presence of two isomeric hepta-O-methyl sucroses establishes that an equilibrium also exists

between two isomeric sucrose monomyristates. The transesterification of sucrose with a methyl ester of a fatty acid, therefore, results in a complex series of equilibrium reactions between sucrose esters with varying degrees of substitution and between isomeric sucrose esters. The equilibrium constant K_e for the reaction

6-myristoyl sucrose $\rightleftharpoons$ 6'-myristoyl sucrose

is given by the equation,

$$K_e = 0.62/0.28 = 2.2,$$

and the difference in standard free energy ($\Delta F°$) between the two isomers is approximately 0.6 kcal $mole^{-1}$ at 80°. The 6'-myristoyl sucrose is thermodynamically more stable than 6-myristoyl sucrose. This difference is probably related to the difference in the non-bonded interactions in the two esters. The pyranose ring of the glucose moiety of sucrose exists in the chair form wherein the atoms on neighbouring carbons in the ring are in the staggered relationship. Therefore, a staggering of the substituents on the 6-carbon with the substituents on the 5-carbon of the glucose molecule will cause an eclipsing of two of the substituents on the 6-carbon with the hydrogen and oxygen on the 4-carbon. This relationship is depicted in the conformational formula I (see Figure XVI). On the other

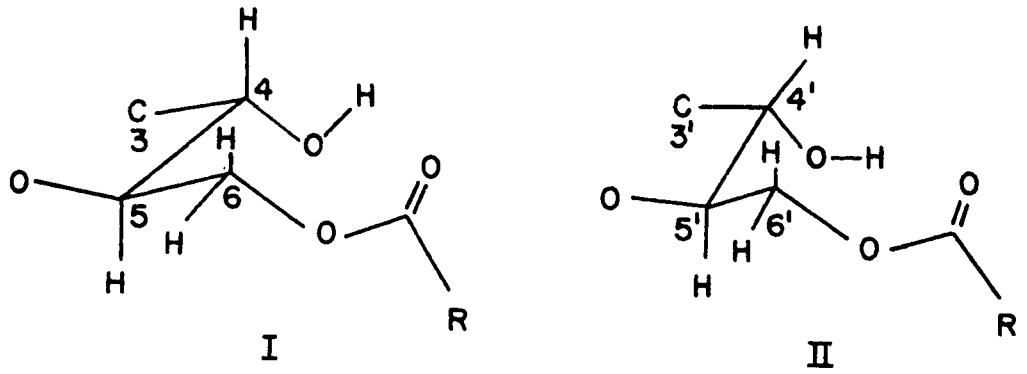


Figure XVI. The conformations of groups or atoms attached to the fourth and fifth carbon atoms in the glucose and fructose moiety of 6-myristoyl and 6'-myristoyl sucrose, respectively.

hand, the atoms on neighbouring carbons in the furanose ring of the fructose moiety can only be in a partially staggered relationship. Therefore, in this case, a staggering of the substituents on carbons 5 and 6 will not eclipse the substituents on the 6-carbon with the hydrogen and the oxygen on the 4-carbon (see conformational formulae II, Figure XVI). Thus, an acyloxy group at the 6'-position of the sucrose molecule will be in a less congested region of the sucrose molecule than an acyloxy group at the 6-position.

It can be concluded moreover, that sucrose dimyristate will occur almost exclusively as 6-,6'-dimyristoyl sucrose.

THE ESTERIFICATION OF 1,4;3,6-DIANHYDRO-D-GLUCITOL

(PART TWO)

ABSTRACT

The reaction of 1,4;3,6-dianhydro-D-glucitol (isosorbide) with an equimolar amount of tosyl chloride in pyridine gave a fourfold greater yield of 5-O-tosyl isosorbide than 2-O-tosyl isosorbide, which have an endo- and exo-tosyloxy group, respectively. 2-O-Tosyl isosorbide reacted about 1.5 times more rapidly with tosyl chloride than did 5-O-tosyl isosorbide. The structures of these compounds were established when it was shown by infrared that only 2-O-tosyl isosorbide had an intramolecular hydrogen bond, consistent with the presence of an endo-5-hydroxyl group, and reacted at least 20 times more rapidly with sodium methoxide than 5-O-tosyl isosorbide. 2-O-Tosyl and 5-O-tosyl isosorbide yielded 1,4;2,5;3,6-trianhydro-D-mannitol and isosorbide, respectively, in reaction with sodium methoxide. The exo-tosyloxy group of 2-O-tosyl 5-O-acetyl isosorbide resists replacement by iodine, whereas the endo-tosyloxy group of 2-O-acetyl 5-O-tosyl isosorbide undergoes facile replacement. The latter compound was recovered unchanged, however, when it was solvolyzed in acetic acid-acetic anhydride containing potassium acetate.

INTRODUCTION

Cope and Shen (87) reported the preparation of a mono-O-tosyl mono-O-acetyl 1,4;3,6-dianhydro-D-glucitol (mono-O-tosyl mono-O-acetyl isosorbide). Since the compound was converted (87) to 1,4;2,5;3,6-trianhydro-D-mannitol on treatment with sodium t-butoxide at reflux temperature for 18 hours, it was concluded that the original compound was 5-O-acetyl 2-O-tosyl isosorbide. In this compound the tosyloxy group is at the exo-2-position in the molecule. However, Cope and Shen (87) had also shown that a transtosylation reaction can take place under these conditions. For example, they showed that when isosorbide was refluxed with sodium t-butoxide in the presence of phenyl p-toluenesulphonate, 1,4;2,5;3,6-trianhydro-D-mannitol was obtained. Recently Jackson and Hayward (88) have shown that the mono-O-acetyl mono-O-tosyl isosorbide, isolated by Cope and Shen, undergoes replacement of the tosyloxy group when heated with sodium iodide dissolved in acetic anhydride. This observation was taken as proof that the compound was 2-O-acetyl 5-O-tosyl isosorbide. In this latter compound the tosyloxy group is at the endo-5-position in isosorbide. Neither of the above experimental methods provided unambiguous proof of the structure of the compound. It is conceivable, for example,

that the endo-acetoxy group of 2-O-tosyl 5-O-acetyl isosorbide could participate in the solvolysis of the tosyloxy group to lead to a 2,5-bridged acetoxonium ion, which could then react rapidly with iodide ion to yield 2-O-acetyl 5-deoxy-5-iodo-1,4;3,6-dianhydro-L-iditol. Evidence for the occurrence of this type of participation in the replacement of a tosyloxy group when a tosylate of a carboxylate ester is treated with sodium iodide in acetone was mentioned on page 159 of this thesis. The The isolation of the latter by Jackson and Hayward (88) could therefore not be taken as unequivocal evidence that the tosyloxy group was at the endo-5-position.

The present investigation was started with a view to obtaining an unambiguous proof of the structure of this compound.

EXPERIMENTAL

Preparation of isosorbide

Isosorbide (II) was prepared by the procedure of Montgomery and Wiggins (89). D-Sorbitol (100 g) was boiled under reflux with concentrated hydrochloric acid (200 ml) for 24 hours. At the end of this period the brown solution was taken to dryness on a rotary evaporator. The syrupy residue was redissolved in water and again evaporated to a syrup. The latter was then distilled at 160-165° at approximately 10 mm pressure, and the light yellow distillate (83 g) crystallized immediately. When the crude distillate was recrystallized from ethyl acetate, isosorbide (44 g) was obtained as long crystalline needles which melted at 62-63.5° with $[\alpha]_D$ + 44.6° (c, 1.78 in water) and +65° (c, 2.94 in pyridine). The reported values are m.p. 61.9-64°, $[\alpha]_D$ + 44.8 (c, 2.22 in water) and 64.9° (c, 2.32 in pyridine) (90).

Reaction of isosorbide with tosyl chloride

Isosorbide (13.2 g; 0.905 moles) was dissolved in anhydrous pyridine (100 ml) and cooled to -5°. An equimolar amount of tosyl chloride (17.2 g) was added and the reaction mixture stored at 5° for 46 hours. Any tosyl chloride present at the end of the period was destroyed by the

addition of distilled water (5 ml) to the reaction mixture. After standing for 15 minutes the reaction mixture was poured into distilled water (250 ml) and extracted with three aliquots (150 ml) of chloroform. The combined chloroform extracts was washed with concentrated hydrochloric acid to remove pyridine and then washed free of acid with distilled water. After drying over anhydrous sodium sulphate the chloroform solution was evaporated under reduced pressure at 50°, to yield a syrupy residue (22.6 g). The latter was dissolved in 95% aqueous ethanol (100 ml), and after the solution was left to stand overnight at 5° a white crystalline compound, m.p. 96-97°, was precipitated. The melting point and infrared spectrum of the substance was consistent with those expected for 2,5-di-O-tosyl isosorbide (91). The filtrate on evaporation under reduced pressure yielded a syrupy residue (17.53 g).

Chromatography of the monotosyl isosorbides on silicic acid (Mallinckrodt).

The syrupy residue (17.53 g) was chromatographed on silicic acid (400 g) which had previously been activated at 120° for 24 hours. The chromatogram was developed with chloroform (4 l.) three fractions were clearly separated in the following order: (a) a white crystalline compound (2.00 g), the melting point and infrared spectrum of which

was identical with that of 2,5-di-O-tosyl isosorbide; (b) a white crystalline compound (III) (3.18 g; 11.7% of theory based on isosorbide), which after recrystallization from ethyl acetate melted at 108-109° with $[\alpha]_D$ + 47.7° (c, 1.86 in chloroform); and (c) a colourless syrup (IV) (12.31 g; 45.4% of theory based on isosorbide), $[\alpha]_D$ + 57.9° (c, 4.27 in chloroform). The total yield of ditosylate was 17.1% of theory.

Infrared analysis of the compounds III and IV

The infrared spectra of the compounds III and IV were both consistent with that expected for a monotosyl isosorbide. A study, using a Beckman DK2 spectrometer, of the infrared spectra in the O-H stretching region of solutions (0.0025 molar) of compounds III and IV in carbon tetrachloride showed absorption at 3564 cm^{-1} and 3624 cm^{-1}, respectively. The latter is characteristic of the stretching vibration of a non-bonded secondary hydroxyl group, whereas the former shows a shift to lower frequency of 60 cm^{-1} which is characteristic of hydroxyl groups forming intramolecular hydrogen bonds (92).

Acetylation of the compounds III and IV

Samples of III (1.74 g) and IV (5.31 g) were acetylated in anhydrous pyridine (5 ml) with acetic

anhydride (5 ml) at 5° for 24 hours. At the end of the reaction period the pyridine and acetic anhydride was removed at 50° under reduced pressure. The residues were dissolved in chloroform (50 ml) and washed successively with concentrated hydrochloric acid, to remove any residual pyridine, sodium bicarbonate solution, to remove any free acid, and finally with distilled water. The individual chloroform extracts were dried, by filtering through filter paper, and then taken to dryness under reduced pressure. The acetate of compound III was isolated as a colorless oil (V) (1.80 g; 90% of theory) with $[\alpha]_D$ + 83.6° (c, 3.43 in chloroform). Compound IV, on the other hand, gave an acetate (I) (5.70 g; 94% theory) which crystallized from methanol in 83% yield and melted at 65.5-66° with $[\alpha]_D$ + 79.2° (c, 3.54 in chloroform). Reported (88) for 2-O-acetyl 5-O-tosyl isosorbide (I), m.p. 64-65° and $[\alpha]_D$ + 77.9° (in chloroform). The nuclear magnetic resonance spectra of compounds I and V were in agreement with those expected for mono-O-acetyl mono-O-tosyl derivatives of isosorbide; furthermore, the infrared spectra were very similar.

Rates of tosylation of compounds III and IV

The rates of tosylation of III and IV were compared by reacting samples of the compounds (0.2256 g,

0.75 mM) with tosyl chloride (2 mM) in anhydrous pyridine (5 ml) at 25°. Aliquots (1 ml) of the solution were taken at intervals and added to 90% pyridine (5 ml) and left standing for 5 minutes. Distilled water (10 ml) was then added and the free acid determined by titration with 0.0882N sodium hydroxide using phenolphthalein as indicator. A sclution of only tosyl chloride in the same molar concentration was used as control.

Assuming second order kinetics the ratio of the rate constants for the two reactions, k_{III}/k_{IV} could be calculated from the equation,

$$k_{III}/k_{IV} = \log b/a(a-c_1)/(b-c_1)/\log b/a(a-c_2)/(b-c_2) \quad (21)$$

where a = the initial concentration of compounds III and IV,

b = " " " of tosyl chloride,

and c_1 and c_2 = the amount of III and IV which has reacted in the same time t.

Since the above equation only involved molar ratios the values for a, b, c_1 and c_2 were expressed in terms of an equivalent volume of 0.0882N sodium hydroxide. The results are given in Table XXIX.

TABLE XXIX

The relative rates of tosylation of compounds III and IV

Time (hours)	a(1)	Volume of 0.0882N NaOH (ml) b	c_1	c_2	k_{III}/k_{IV}
1	1.7	8.84	0.29	0.24	1.24
6	1.7	8.88	1.00	0.80	1.41
16	1.7	8.86	1.46	1.12	1.88
28(2)		8.86	1.67	1.31	
	Average value for k_{III}/k_{IV}				1.51

1. Since a 1 ml aliquot of the reaction solution contains 0.15 mM of compounds III and IV a would be equivalent to a titration of 1.7 ml of 0.0882N sodium hydroxide.
2. The ratio of k_{III} to k_{IV} was not calculated since 98% and 77% of compounds III and IV, respectively had reacted with tosyl chloride.

Relative rates of reaction of compounds III and IV with sodium methoxide

Samples of compounds III (0.3450 g; 1.15 mM) and IV (0.2905 g; 0.97 mM) were dissolved in 0.2N sodium methoxide solution (10 ml) and left standing at room

temperature. At intervals over a period of 72 hours, aliquots (1 ml) were taken and titrated with 0.064N hydrochloric acid. At the end of the reaction period, III had consumed 1.08 milliequivalents (94% theory) and IV 0.065 milliequivalents (17% theory) of sodium methoxide. Assuming second order kinetics the ratio of the rate constants k_{III}/k_{IV} was calculated from equation 21 where

a = the initial concentrations of compounds III and IV,

b = " " concentration of sodium methoxide,

and c_1 and c_2 = the amount of compounds III and IV which had reacted in the same time t.

The results are given in Table XXX.

TABLE XXX

The relative rates of reaction of compounds III and IV[1] with sodium methoxide

Time (hours)	c_1 (mM)	c_2 (mM)	k_{III}/k_{IV}
24	0.467	0.013	$\approx$ 30
48	0.932	0.128	$\approx$ 14
72	1.082	0.166	$\approx$ 20
	Average k_{III}/k_{IV}		$\approx$ 20

1. The initial concentration of compounds III and IV were 1.15 mM and 0.97 mM respectively, and the corresponding concentration of sodium methoxide was 1.92 mM in 10 ml of solution.

Reaction of compounds III and IV with sodium methoxide under reflux

Compounds III (0.2005 g; 0.667 mM) and IV (0.4585 g; 1.53 mM) were refluxed with 2 ml and 4 ml, respectively, of methanol approximately 1N in sodium methoxide for 8 hours. After cooling to room temperature, the excess sodium methoxide was neutralized with solid carbon dioxide. The reaction mixtures were taken to dryness at room temperature under reduced pressure and the residues extracted with ether and acetone, respectively. After solvent removal, the residues were distilled under reduced pressure. Compound III yielded a crystalline compound (0.0689 g; 81% theory), m.p. 68-68.6° and $[\alpha]_D$ + 127.9° (c, 1.38 in water), the infrared spectrum of which showed no absorption in the O-H stretching or aromatic skeletal vibration regions and was consistent with that expected for 1,4;2,5;3,6-trianhydro-D-mannitol (VI). The latter was reported (87) to melt at 66.5-67.2 with an $[\alpha]_D$ + 128.4° (c, 4.2 in water). On the other hand, IV yielded a crystalline compound (0.1455 g; 70% theory) whose infrared spectrum was identical to that of isosorbide (II).

Reaction of compounds I and V with sodium iodide

The compounds V (1.43 g; 4.2 mM) and I (3.43 g; 10 mM) were refluxed with 20 ml and 50 ml, respectively, of

a 0.5M solution of sodium iodide in acetic anhydride. After 150 minutes the solutions were cooled to room temperature and the sodium p-toluenesulphonate was recovered by filtration and weighed as described by Jackson and Hayward (90). Compound V yielded 0.060 g (7.4% theory) and I yielded 1.78 g (92% theory) of sodium p-toluenesulphonate. The filtrates were taken to dryness under reduced pressure and the residues dissolved in chloroform (50 ml). The chloroform solutions were washed with sodium thiosulphate solution and then distilled water, and then dried by anhydrous sodium sulphate. Compound V was recovered unchanged in 70% yield, and a yellow oil (2.60 g) in 87% yield from the two reactions, respectively. The latter would not crystallize and had an $[\alpha]_D$ + 95.6° (c, 0.51 in chloroform). Jackson and Hayward had reported 2-O-acetyl 5-deoxy-5-iodo-1,4;3,6-dianhydro-L-iditol (88) had an $[\alpha]_D$ + 100.5° (c, 0.83 in chloroform).

Solvolysis of compound IV in acetic acid-acetic anhydride

Compound IV (1.20 g) was refluxed for 24 hours in an acetic acid-acetic anhydride mixture (4:1)containing potassium acetate (1 g). At the end of this period the solvent was removed under reduced pressure and the residue was extracted with ether and the ether solution filtered. Removal of the solvent gave a solid material, which was

recrystallized from methanol to yield a crystalline compound (1.12 g; 81% theory) which showed no depression in melting point when admixed with compound I.

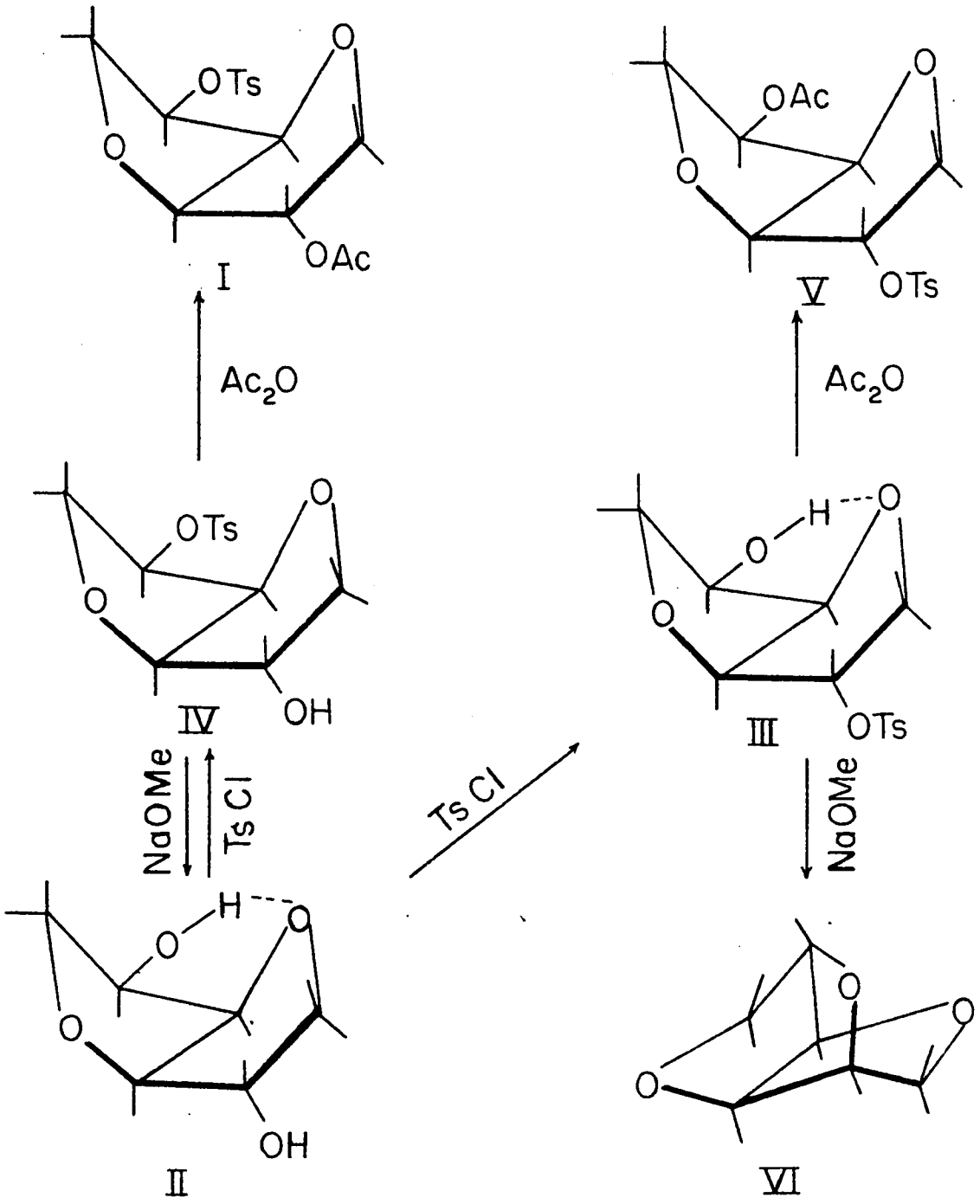


Figure XVII. Reactions which established the structures of the isomeric mono-O-tosyl mono-O-acetyl derivatives of isosorbide.

DISCUSSION

All of the reactions which were carried out to establish the structures of the monotosyl esters of isosorbide are shown graphically in Figure XVII.

When isosorbide (II) was reacted with an equimolar amount of tosyl chloride in pyridine at 5° for 40 hours (87, 88), the ditosyl derivative was obtained in 17.1 percent yield. Chromatography of the residue on silicic acid, with chloroform as a developing phase gave a crystalline monotosylate (III) in 11.7 percent yield, and an isomer (IV), which has resisted crystallization, in 45.4 percent yield. The structures of the monotosylates III and IV were readily established through their behaviour with sodium methoxide. Compound III consumed the base about 20 times more rapidly than IV at room temperature and led to the formation of 1,4;2,5;3,6-trianhydro-D-mannitol (VI). On the other hand, compound IV when heated in the presence of sodium methoxide was converted to isosorbide (II) in excellent yield. Clearly, therefore, compounds III and IV were 2-O-tosyl isosorbide and 5-O-tosyl isosorbide, respectively (see Figure XVII). This conclusion was supported by the fact that the compounds III and IV showed absorption maxima for the hydroxyl groups, in the infrared, at 3564 cm^{-1} and 3624 cm^{-1}, respectively, when 0.0025M in carbon tetrachloride. These results indicate

intramolecular hydrogen bonding (92) in compound III which can only be possible with the hydroxyl groups at the endo-5-position. Acetylation of compound IV yielded crystalline 2-O-acetyl 5-O-tosyl isosorbide (I). This compound was identical to that isolated by Cope and Shen (87) and clearly disproves their claim that the compound was 2-O-tosyl 5-O-acetyl isosorbide. Acetylation of the crystalline monotosylate (III) gave 2-O-tosyl 5-O-acetyl isosorbide (V) which has not crystallized. When this compound was reacted with sodium iodide in acetic anhydride for 150 minutes the starting material was recovered unchanged. Consequently, the acetoxy group of compound V is not well disposed for participation in the dissociation of the tosyloxy group. On the other hand, 2-O-acetyl 5-O-tosyl isosorbide (I) undergoes facile replacement of the tosyloxy group by iodine (88) under the same conditions. Compound I, moreover, resisted refluxing in 4:1 acetic acid-acetic anhydride containing potassium acetate for 24 hours.

A consideration of a molecular model of isosorbide (II) shows that the endo-5-hydroxyl is almost eclipsed with the oxygen atom which bridges the 1- and 4-positions, whereas the exo-2-hydroxyl is eclipsed only with hydrogen atoms. The non-bonded interaction between the eclipsed oxygen atoms should be especially unfavourable since it

brings into close opposition carbon-to-oxygen bonds polarized in the same direction. This contention is supported by the fact that D-glucitol (sorbitol) is converted more readily to the 1,4-anhydride which possesses a 2,3-trans-glycol, than to the 3,6-anhydride which possesses a 4,5-cis-glycol (91). It could be anticipated that the endo-5-hydroxyl group of isosorbide would be intramolecularly hydrogen bonded to the eclipsing 4-oxygen atom. This conclusion was supported by the observation (see above) that the endo-5-hydroxyl group of 2-O-tosyl isosorbide (III) was in fact involved in an intramolecular hydrogen bond. Therefore, one might expect (87) that the exo-2-hydroxyl group of isosorbide (II) would undergo tosylation substantially more rapidly than the sterically shielded and hydrogen bonded endo-5-hydroxyl.

A study of the rates of tosylation of 2-O-tosyl and 5-O-tosyl isosorbide, however, showed that the former compound reacted approximately 1.5 times faster than the latter. This means that the sterically hindered, hydrogen bonded, endo-5-hydroxyl group is more reactive to acylation than the exo-2-hydroxyl group. This phenomena has also been observed by Mattok and Lemieux in the reaction of isosorbide with benzoyl chloride in pyridine. These workers (93) have shown that the reaction follows first order kinetics in isosorbide, benzoyl chloride and pyridine,

and that the thermodynamically less stable endo-5-benzoate was formed preferentially. Furthermore, the greater steric requirements for the acylation of the endo-5-hydroxyl group was reflected in a more negative entropy of activation (93). These results may appear to suggest that the difference in reactivity of the two hydroxyl groups in isosorbide (II) is in some way related to the fact that one of the hydroxyl groups (the endo-5-hydroxyl) is hydrogen bonded. However, evidence has recently been obtained (93) which indicates that intramolecular hydrogen bonding does in fact, as would be expected, decrease the reactivity of the hydroxyl groups toward esterification with an acyl halide in pyridine. The present results therefore suggest that the endo-5-hydroxyl group is quasi-equatorially oriented as shown in I and is more reactive than the exo-2-hydroxyl group in spite of the intramolecular hydrogen bonding because it is sterically more favourably oriented for reaction than the quasi-axial 2-hydroxyl group. It is well known that hydroxyl groups which are equatorially orientated in a six numbered ring (in the chair conformation) are acylated more rapidly than hydroxyl groups which are in the axial orientation (94,95). The above conclusion clearly indicates the hazard of considering 5-membered rings to exist in planar conformations as was assumed by Cope and Shen (87).

CLAIMS TO ORIGINAL RESEARCH

1. Analytical methods were developed to measure the rate of transesterification of sucrose with the methyl ester of a fatty acid, using a base as catalyst; to obtain the yield of sucrose ester at the end of the reaction period, and to determine the composition of the sucrose ester.
2. It was established that reproducible rates of reaction, yields of sucrose ester and product composition were only obtained when the reaction was carried out under conditions for homogeneous catalysis.
3. The transesterification reaction was shown to be a bimolecular reaction following first order kinetics in sucrate ion and methyl ester, respectively.
4. It was established that the rate of transesterification was independent of the sucrose concentration, and the cation of the base used to catalyze the reaction. The chain length of the fatty acid (C_{14}-C_{18}) also had little effect on the rate of reaction.
5. The rate of transesterification increased in a non-linear manner with increasing potassium sucrate concentration, except at concentrations of the latter less than 3×10^{-3} molar.

6. The rate of transesterification approximately doubled for a 17° increase in the reaction temperature, and the activation energy and frequency factor for the reaction were determined. Approximate values for the thermodynamic constants ΔF^{+}, ΔH^{+} and ΔS^{+} were also determined.

7. It was established that our standard transesterification conditions gave essentially quantitative yields of sucrose ester in 2-1/2 hours at 80° and 1-1/2 hours at 97°.

8. An analytically pure sample of sucrose monomyristate, and a sample of sucrose dimyristate in a high state of purity, was isolated.

9. The equilibrium constants for the major equilibrium reactions taking place in the transesterification reaction solution were determined.

10. It was established that only the thermodynamic product was obtained in the transesterification reaction, and that a complicated series of equilibria exist between sucrose esters with varying degrees of substitution and between isomeric sucrose esters.

11. It was established that water had an adverse effect on the rate of transesterification and yield of sucrose ester, but no side reactions occurred if the reaction conditions were anhydrous.

12. A mathematical equation was developed which enabled the yield of sucrose monomyristate to be predicted for any molar ratio of sucrose to methyl myristate.

13. The structure of sucrose monomyristate was shown to be almost exclusively 6- and 6'- myristoyl sucrose with the latter being present in higher concentration.

14. Two isomeric mono-O-tosyl isosorbides were isolated for the first time and their structures unambiguously assigned. The structures of the corresponding isomeric mono-O-tosyl mono-O-acetyl isosorbides were also established.

15. 2-O-Tosyl and 5-O-tosyl isosorbide gave 1,4;2,5;3,6-trianhydro-D-mannitol and isosorbide, respectively, when reacted with sodium methoxide.

16. The endo-5-hydroxyl of isosorbide reacted with p-toluenesulphonyl chloride, in anhydrous pyridine, 1.5 times more rapidly than the exo-2-hydroxyl group.

17. It was established that the acetyl group of 2-O-tosyl 5-O-acetyl isosorbide did not participate in the replacement of the tosyloxy group with iodine.

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