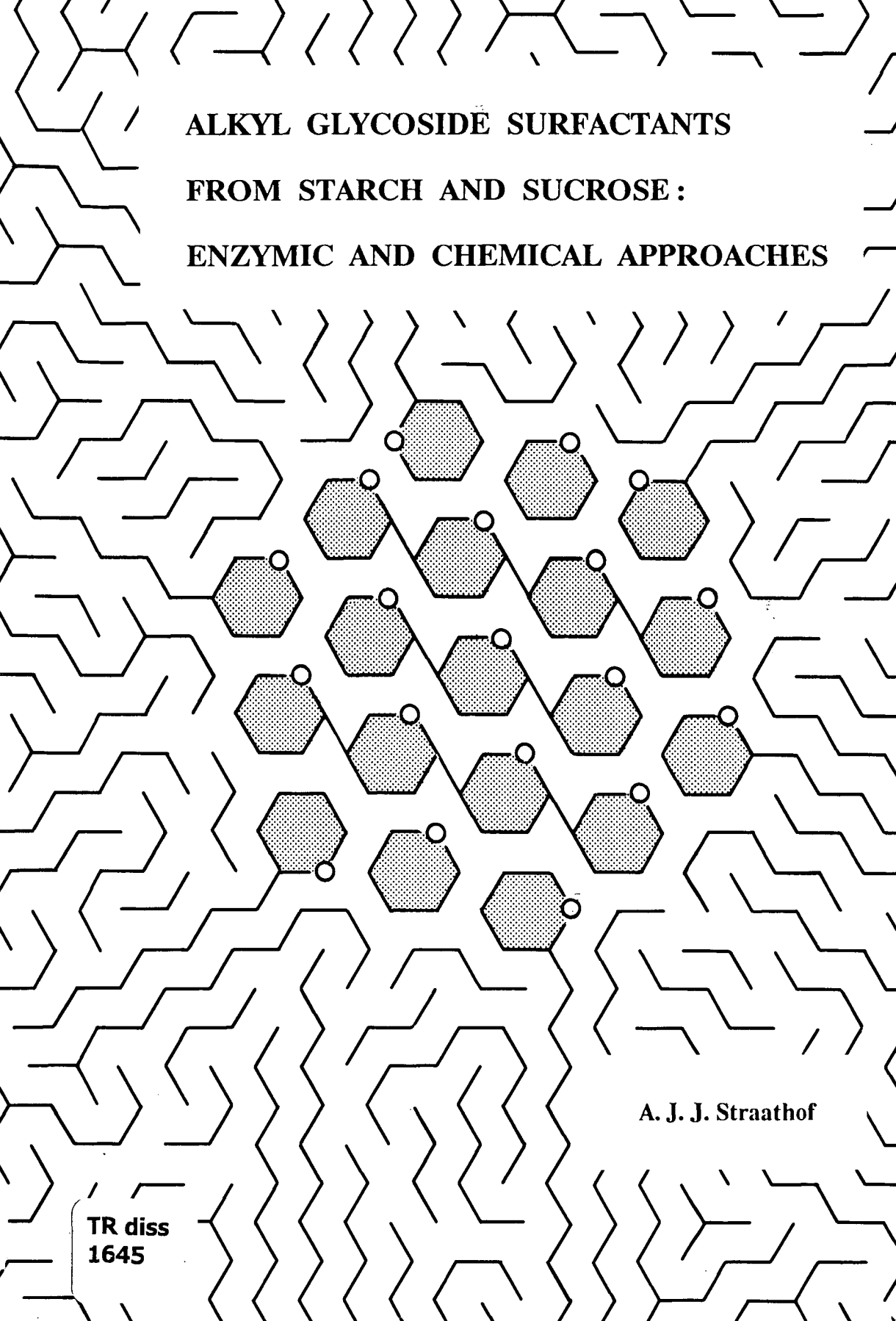
ALKYL GLYCOSIDE SURFACTANTS FROM STARCH AND SUCROSE: ENZYMIC AND CHEMICAL APPROACHES



A. J. J. Straathof

ALKYL GLYCOSIDE SURFACTANTS FROM STARCH AND SUCROSE: ENZYMIC AND CHEMICAL APPROACHES

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus,
prof.dr. J.M. Dirken,
in het openbaar te verdedigen
ten overstaan van een commissie aangewezen
door het College van Dekanen
op donderdag 16 juni 1988 te 16.00 uur

door

Adrianus Johannes Jozef Straathof
geboren te 's-Gravenhage
scheikundig ingenieur

Dit proefschrift is goedgekeurd door de promotoren
Prof. dr. ir. A.P.G. Kieboom
en
Prof. dr. ir. H. van Bekkum

STELLINGEN

1. De indertijd door de firma Rohm & Haas Co. geclaimde bereidingswijze voor dodecyl-α-D-glucopyranoside leidt, gezien de vermelde $[\alpha]_D$-waarde en faseovergangstemperaturen, tot een mengsel van α- en β-anomeren.

   Rohm & Haas Co., Neth. Appl. 6,409,068 (1965); Chem. Abstr., **63** (1965) 4518g.

2. Op basis van de evenwichtskonstanten van de door sucrosefosforylase, maltosefosforylase en glucoamylase gekatalyseerde reacties kan voor de hydrolyse van sucrose bij ~ 30°C een evenwichtskonstante (K = [glucose]·[fructose]/[sucrose]) van ~ 15000 berekend worden.

   A. Guibert, Thèse, Toulouse, 1983, p.113.
   R. van Tilburg, Proefschrift, Delft, 1983, p.140.

3. De discrepantie tussen de door Gorin en Mazurek op basis van de kristalstructuur onmogelijk geachte en door Ponomarenko en Lapenko aangetoonde boraatestervorming van 1,6-anhydro-β-D-glucopyranose kan verklaard worden doordat de auteurs water resp. aceton als oplosmiddel gebruiken.

   P.A.J. Gorin en M. Mazurek, Can J. Chem., **51** (1973) 3277.
   E.Yu. Ponomarenko en V.L. Lapenko, Zh. Obsh. Khim., **49** (1979) 224.

4. De waarde van de theorie van Shearwin en Winzor, die de daling van de invertase-activiteit bij hoge sucroseconcentratie verklaren op basis van thermodynamische niet-idealiteit, is niet duidelijk omdat de auteurs geen experimenten hebben verricht in het interessante concentratiegebied van 0,9-2,3 M sucrose.

   K.E. Shearwen en D.J. Winzor, Arch. Biochem. Biophys., **260** (1988) 532.
   A.J.J. Straathof, A.P.G. Kieboom en H. van Bekkum, Carbohydr. Res., **146** (1986) 154.

5. Om te kunnen beoordelen in hoeverre de veronderstelling van Klibanov "that most, if not all, enzymes can work in organic solvents" juist is,

is openbaarmaking van *alle*, dus ook negatieve, resultaten betreffende enzymen in organische oplosmiddelen gewenst.

A.M. Klibanov, CHEMTECH, (1986) 354.

6. De door Davies toegekende HLB-groepswaarden zijn gebaseerd op een klein aantal experimenteel bepaalde HLB-waarden en daardoor beperkt toepasbaar. Het feit dat de groepswaarden toch veelvuldig gebruikt worden, wijst op een duidelijke behoefte om emulsiestabiliteit te kunnen voorspellen op basis van de chemische structuur van de emulgator.

   J.T. Davies, Proc. 2nd Int. Congr. Surf. Act., J.H. Schulman (Ed.), Londen, 1957, vol. 1, p.426.

7. In de koolhydraatchemie wordt weinig aandacht geschonken aan het feit dat beschermende groepen tevens de oplosbaarheid van koolhydraten bevorderen in apolaire organische oplosmiddelen.

8. In petroleum ether 40-60 is het aandeel van componenten met een kookpunt tussen 40 en 60°C in de regel bescheiden.

9. Het gebruik van de computer leidt in wetenschappelijk onderzoek niet altijd tot tijdwinst omdat het vaak een impuls geeft tot een nauwkeurigere bestudering van het te onderzoeken onderwerp.

10. Sommige bezwaren tegen het loslaten van wolven in Nederlandse natuurreservaten vertonen een pijnlijke overeenkomst met de huidige gevolgen van de opkomst van de pitbullterrier.

A.J.J. Straathof
16 juni 1988

Aan mijn ouders

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CHAPTER 1

INTRODUCTION

1.1 CARBOHYDRATES AS RENEWABLES

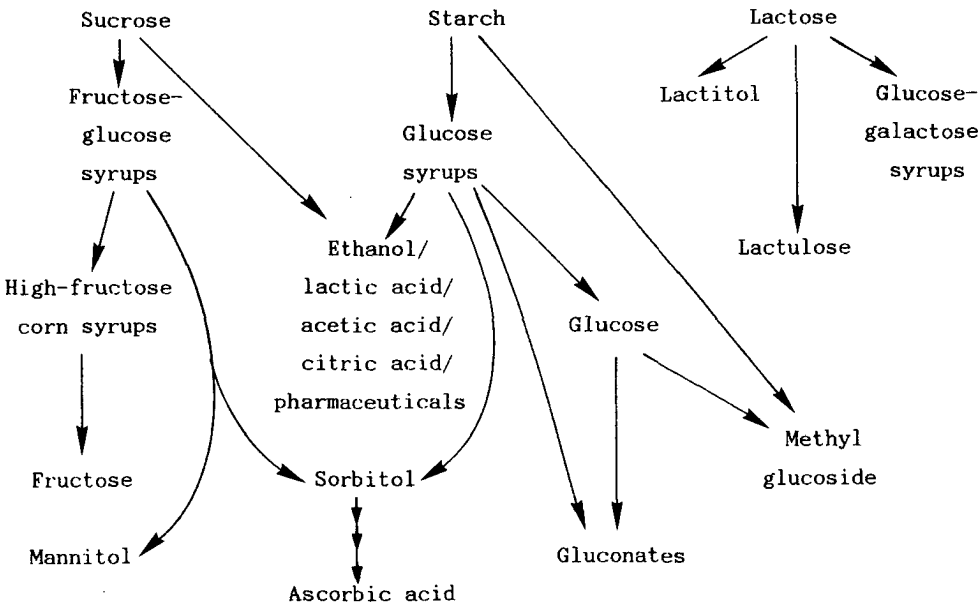
As a source of raw material for organic chemical products, mineral oil and natural gas will be available in sufficient quantity for several decades. Nevertheless, on the long term these fossil feedstocks are limited and renewables such as carbohydrates will become more and more important. Presently, substantial surplusses of carbohydrates such as starch and sucrose are produced, asking for the development of new chemical processes based on these renewables.

A partial switch to the use of carbohydrates as a raw material will not only be determined by their price and availability but also by the development of appropriate technologies for their selective conversion into well-defined chemical products. Some important mono- and disaccharides and derivatives thereof that are presently produced are shown in Scheme 1. Chemo-catalytic, enzymic, and fermentative processes are used. It should be noted that all compounds in Scheme 1 are non-toxic, which permits their application in food products. An additional advantage of most carbohydrate derivatives is their ready biodegradation, which is of importance from an environmental point of view.

In the past decades, several product markets have been considered to be accessible to sucrose[1] and starch[2] derivatives. An attractive and potentially large outlet for carbohydrates is the production of carbohydrate-derived surfactants.

1.2 SURFACTANTS[3,4]

The term surfactant is a contraction of the longer term surface active agent. The most fundamental characteristic of surfactants is the presence of groups of opposing solubility tendencies within a molecule, typically a



Scheme 1. Routes for the production of low-molecular carbohydrate derivatives. Starting materials not shown are hemicellulose (for furfural and xylitol) and molasses (for fermentation). Only a limited number of fermentation products has been included.

hydrophilic ionic, polyoxyethylene, or polyol group, and a hydrophobic hydrocarbon chain. Consequently, surfactant molecules form oriented monolayers at phase interfaces. In solution, surfactants form aggregates (micelles) when their concentration exceeds a certain limiting value[5], the critical micelle concentration (CMC). This behaviour of surfactant molecules explains their cleaning, foaming, wetting, emulsifying, solubilising, and dispersing properties.

Although surfactants are well-known for their cleaning properties (detergency), the range of applications is much wider (Table 1). It should also be noted that pure surfactants have liquid crystalline properties[7].

The area of application for a surfactant can be anticipated from its aqueous solubility (Table 2). Dimensionless numbers can be assigned to surfactants according to the Hydrophile-Lipophile Balance (HLB) scale, but since the HLB is not well-defined widely different values may be obtained for a single surfactant using different methods[8-9]. Moreover, Rosen[10-11]

explained that in comparing the performance of different surfactants it is necessary to distinguish between the *efficiency* of the surfactant (*i.e.* the

Table 1. Surfactant consumption in Western Europe, Japan, and USA in 1982 (ref. 6).

Area of application	(10^3 t)	Share (%)
Washing and cleaning		42
Cosmetics and pharma		7
Textile and fiber		17
Leather and f	50	1
Paint, plast	200	4.5
Cellulose- and	100	2
Mining, flotation	300	7
Metal industry	130	3
Building industry	50	1
Pesticides	100	2
Food industry	200	4.5
Other applications	400	9
Total	4480	100

Table 2. Relation between aqueous solubility, type of application, and HLB-value (ref. 8).

Aqueous solubility	Type of application	HLB-range
ill-soluble	W/O-emulsifier	3-6
milky dispersion	wetting agent	6-8
stable dispersion	wetting agent and O/W-emulsifier	8-10
translucent to clear solution	O/W-emulsifier	10-13
clear solution	detergent	13-15

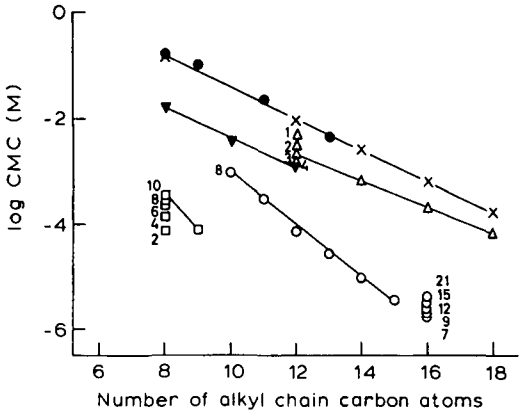


Fig. 1. Critical micelle concentration of some surfactants as a function of the number of alkyl chain carbon atoms. ●, Sodium soaps (20-40 °C, ref. 12); ×, Sodium alkyl sulfates (40 °C, ref. 13); ▼, Sodium p-alkylbenzene sulfonates (35-60 °C, ref. 14); Δ, Sodium alcohol ether sulfates (40 °C, refs. 15-16); o, Alcohol ethoxylates (25 °C, refs. 17-18); □, p-Alkylphenol ethoxylates (25 °C, refs. 19-20). The number of oxyethylene moieties is given in the graph.

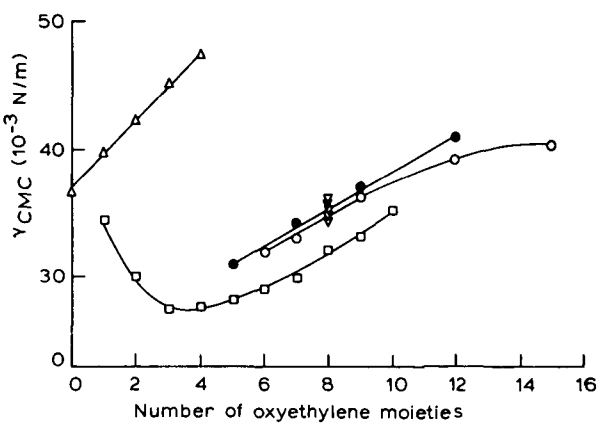
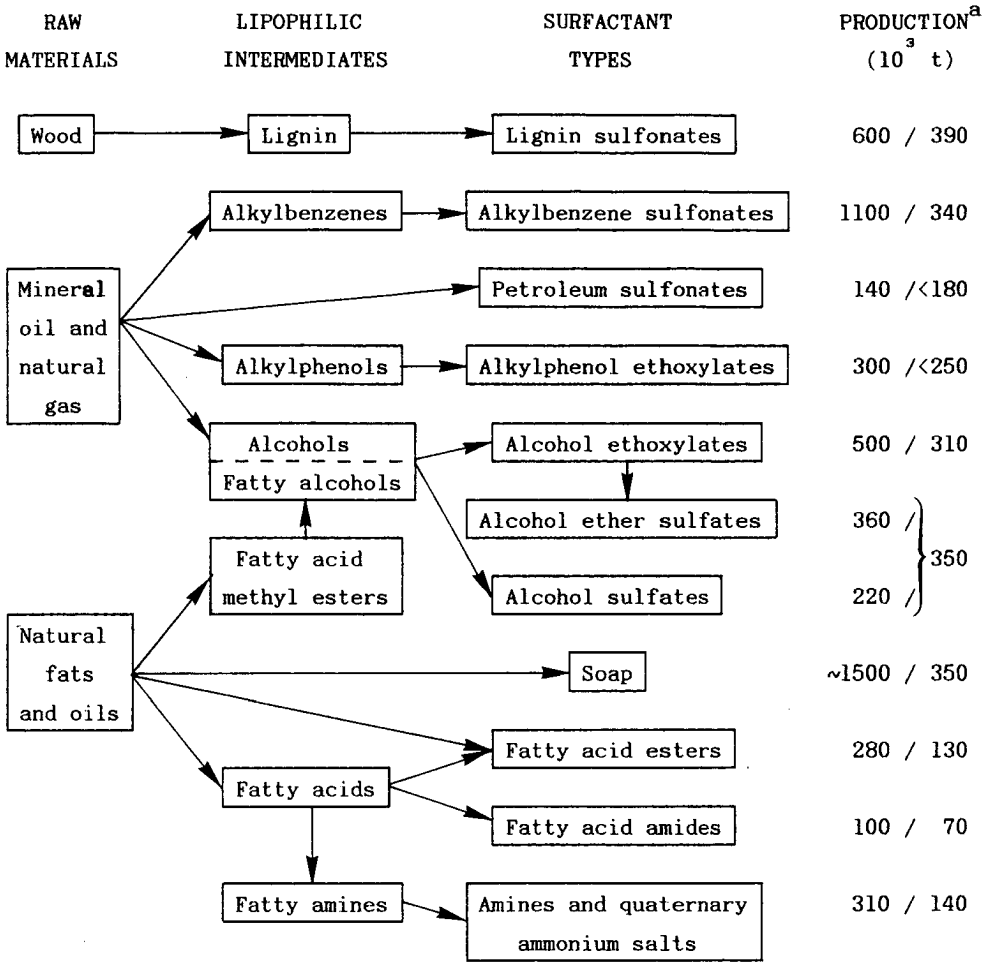


Fig. 2. Surface tension of some surfactants at the CMC as a function of the number of oxyethylene moieties. Δ, Sodium dodecanol ether sulfates (40 °C, ref. 16); ●, Dodecanol ethoxylates (25 °C, ref. 21); o, Hexadecanol ethoxylates (25 °C, ref. 21); ∇, Decanol (upper) to tetradecanol (lower) octaethoxylates (25 °C, ref. 18); □, p-Octylphenol ethoxylates (25 °C, ref. 19).



Scheme 2. Synthetic routes leading to the major surfactant types.
[a] *Surfactant production in US, Western Europe, and Japan in 1982 (first number, ref. 6) and in US in 1986 (second number, ref. 22).*

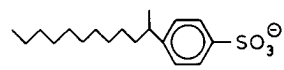
amount required to produce a certain effect) and its *effectiveness* (*i.e.* the *maximum effect* that the surfactant can produce). The efficiency was expressed as pC_{20} (*i.e.* the concentration required for 20 × 10^{-3} N/m surface tension reduction) and the effectiveness as a_m (*i.e.* the maximum surface area per surfactant molecule)[8]. Alternatively, the CMC and the surface tension at the CMC (γ_{CMC}) may be useful quantities since micelles are

required for *e.g.* detergency and solubilisation, and a low γ_{CMC} indicates high surface activity. At concentrations above the CMC no significant decrease in surface tension occurs.

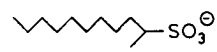
Fig. 1 indicates that the CMC is largely dependent on the ionogenity and the alkyl chain length of the surfactant molecule (a benzene ring counts for $3^1/_2$ alkyl carbon atoms). Fig. 2, on the other hand, shows that for a number of oxyethylene-containing surfactants for which reliable data were found γ_{CMC} is primarily dependent on the structure of the hydrophilic group. These relationships thus allow a prediction of the performance of a surfactant from its molecular structure.

Scheme 2 shows synthetic routes leading to the major surfactant types. Representative structures are given in Scheme 3. Lignin sulfonates, which do

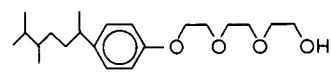
Alkylbenzene sulfonate



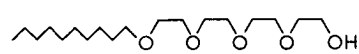
Petroleum sulfonate



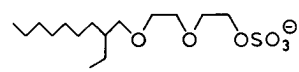
Alkylphenol ethoxylate



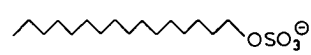
Alcohol ethoxylate



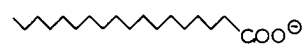
Alcohol ether sulfate



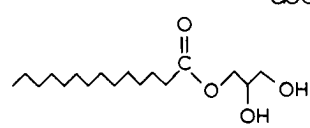
Alcohol sulfate



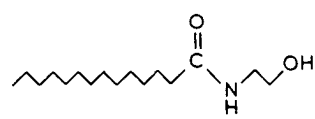
Soap



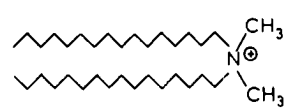
Fatty acid ester



Fatty acid amide



Quaternary ammonium salt



Scheme 3. Structure of major surfactant types.

not form oriented monolayers or micelles, are not included. The hydrocarbon chains are derived either from crude oil (through cracking, ethylene polymerisation, and linear olefin functionalisation, and n-paraffin functionalisation) or from natural fats and oils. Hydrophilic groups are introduced predominantly by reaction with sulfur trioxide or ethylene oxide. Numerous other types of surfactants have been devised, and several hundreds of them are produced commercially[3]. Commercial surfactants consist of a mixture of homologues with a relatively narrow range of CMC and γ_{CMC} values.

1.3 CARBOHYDRATE-DERIVED SURFACTANTS

The literature on long-chain derivatives of mono- and oligosaccharides has been covered until 1960 by Ames[23]. The present review will be restricted to surfactants that have been prepared in a limited number of reaction steps (thus without the use of protecting groups) from the carbohydrates of Scheme 1 and the lipophilic intermediates of Scheme 2. Microbial surfactants are also shortly dealt with.

Anhydrosorbitol esters

Heating sorbitol and fatty acids in the presence of an acidic catalyst yields anhydrosorbitol esters by formation of internal ethers and esterification. Mono-, di-, and triesters of 1,4-anhydrosorbitol and mono- and diesters of 1,4;3,6-dianhydrosorbitol are the principal products[3]. Anhydrosorbitol esters are commercially produced in at least ten countries[24]. They are applied as emulsifiers and solubilizers in food, beverages, pharmaceuticals, synthesic fiber manufacture, textile processing, and cosmetic products. Subsequent oxyethylation of anhydrosorbitol esters leads to a series of more hydrophilic surfactants. In 1980, US production of anhydrosorbitol esters and their oxyethylated derivatives was 13,200 and 11,900 t, respectively[3].

Sucrose esters

Mixtures of mono- to triesters of sucrose are obtained by potassium carbonate-catalysed transesterification of fatty acid methyl esters or triglycerides in aprotic polar solvents such as DMF or *N*-formylmorpholine[25-28]. In this way sucrose is smoothly converted, but these solvents are expensive, toxic, and difficult to remove. As an alternative, water,

methanol, and propylene glycol have been reported as the reaction medium[26-27]. Solvent-free methods, using potassium soaps or sucrose esters as emulsifiers have also been claimed[27,29], as well as numerous other variations of the reaction procedure. Extensive purification was required for all processes.

Sucrose esters are produced commercially in Japan, France, the UK, and the FRG[24]. They have been approved for food applications in most Western countries. In these applications, the FDA has dictated low limit values for solvent contaminations, *e.g.* ⩽ 10 ppm for isobutanol and ⩽ 2 ppm for DMSO[30]. Food, pharmaceutical, and cosmetic uses seem to be growing steadily.

Alkyl oligoglucosides

The acid-catalysed conversion of D-glucose in fatty alcohols yields mixtures of alkyl oligo-D-glucosides having an undefined oligoglucoside structure. Because of the limited solubility of D-glucose in fatty alcohols, the reaction has also been carried out via the methyl and butyl D-glucosides. Water, methanol, butanol, and excess fatty alcohol have to be removed in these processes[4,31-32].

The number of patents on the preparation and application of alkyl oligoglucosides has grown progressively since 1983 (Fig. 3). In 1988, a plant for the production of alkyl oligoglucosides ("alkyl polyglycosides") will be built in the US[33]. The surfactant properties of these products are comparable to those of alcohol ethoxylates. They are also reported to be nontoxic, mild to the skin, and completely biodegradable[34].

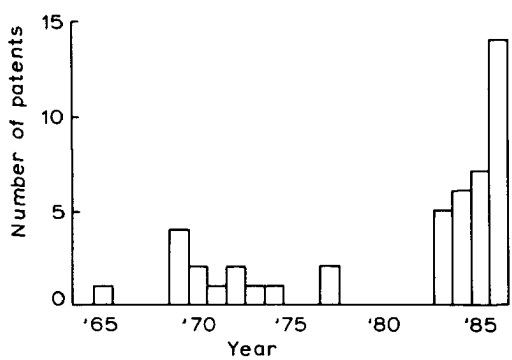


Fig. 3. Annual number of patents concerning the preparation and application of alkyl (oligo)glucosides, indexed in Chemical Abstracts.

Other carbohydrate-derived surfactants

Lactitol has been successfully esterified with fatty acid salts[35], yielding mixtures of mono-, di-, and triesters. Reaction of D-glucose with acyl halides yielded mainly 6-esters[36], whereas with lactose mixtures of lactose fatty acid esters were obtained[37]. 1-Esters of D-glucose, lactose, and maltose have been prepared by reaction with acylated heterocycles such as 1-stearoylimidazole[38,39].

The reaction of D-glucose or sucrose with alkyloxymethyl chloride gave mixtures of alkyloxymethyl ethers with significant surface activity[40,41]. Carbohydrate ethers have also been prepared by the reaction with alkyl glycidyl ethers[42]. Mixtures of carbonates of glucose have been obtained by the reaction with alkyloxycarbonyl chloride[43].

Amide surfactants have been prepared from either aldonic acids and alkylamines[44] or from aminopolyols (obtained by reductive amination of reducing sugars) and fatty acids anhydrides or halides[45,46]. Surfactants obtained by the hydrogen chloride-catalysed reaction of fatty amines with glucose, fructose, lactose, and sucrose showed insufficient stability[47].

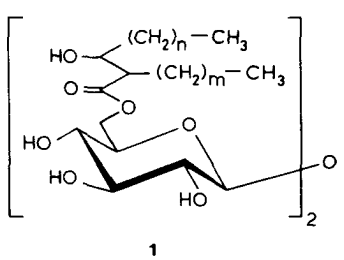
Anionic surfactants have been prepared by the reaction of 2-acyl-1,4; 3,6-dianhydrosorbitol with sulfur trioxide[48] or by the reaction of α-sulfo fatty acid chlorides or methyl esters with sucrose or hexitols[49].

Microbial surfactants

Examples of microbial surfactants include glycolipids, such as trehalose ester (1) and emulsan biopolymer (2)[50]. They can be produced by microbial fermentation of wastes[51]. Because of their oil-emulsifying ability they have been tested for application in enhanced oil recovery, in oil pollution removal, and in heavy oil transportation[52-53].

Concluding remarks

Carbohydrate-derived surfactants will be readily biodegradable, an important property for environmental reasons, so that they have the potential to replace more and more oil-based surfactants. Furthermore, carbohydrate-derived surfactants are generally regarded as safe (GRAS standard) because of their low skin and oral toxicity. Their application has extended particularly in the food, pharmaceutical, and cosmetic sectors. Residues of toxic solvents have to be removed thoroughly from these surfactants, or, preferably, the use of toxic solvents has to be avoided in



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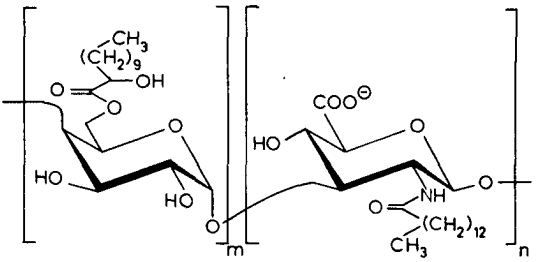


2

the production processes. The opposing solubility tendency of the starting compounds (carbohydrate *vs.* long-chain alkyl compound), however, is troublesome in this respect.

Comprehensive data relating the surface activity of carbohydrate-derived surfactants with their structure are relatively scarce. Generally, γ_{CMC} is well below 40 × 10^{-3} N/m, but the data are not always consistent. On the other hand, Fig. 4 shows that the CMC of carbohydrate-derived surfactants is

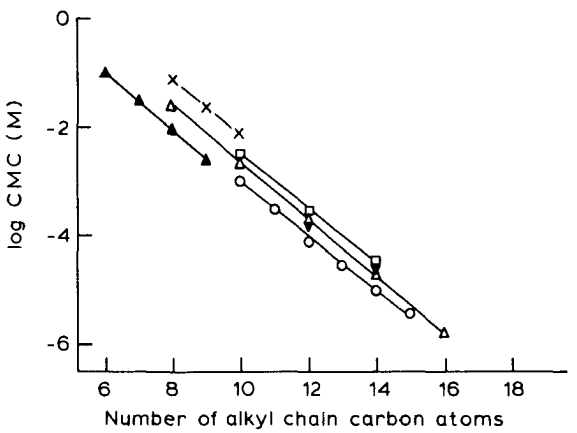


Fig. 4. Critical micelle concentration at 25°C for some carbohydrate-derived surfactants as compared to alcohol octaethoxylates (o). Δ, *Alkyl β-D-glucopyranosides (ref. 54);* ▲, *Alkyl β-D-thioglucopyranosides (ref. 55);* ▼, *Alkyl β-maltosides (ref. 56);* □, *N-Alkyllactobionamides (ref. 44);* ×, *N-Alkanoyl-N-methylglucamines (ref. 57).*

comparable with those of alcohol ethoxylates with the same alkyl chain length (*cf.* Fig. 1).

It has to be noted that the reactions applied to the commercial production of carbohydrate-derived surfactants generally yield mixtures of compounds having widely different properties, *e.g.* sucrose monopalmitate and dipalmitate with interfacial tensions of $\sim 13 \times 10^{-3}$ N/m and $\sim 1 \times 10^{-3}$ N/m, respectively[9]. Work-up procedures for these reaction mixtures are inherently complicated by the inclination of surfactants to concentrate at phase boundaries. More selective reactions or separation techniques are required to obtain well-defined carbohydrate-derived surfactants so that efficiency and effectiveness can be matched better to a required performance.

In addition, possible application of surfactants as liquid crystals[58] asks for compounds of high purity.

1.4 SCOPE OF THIS THESIS

With starch (glucose) and sucrose as starting materials, a number of principally different conversion procedures towards *O*-alkylated carbohydrates have been investigated.

Scheme 4 shows the reaction routes that are dealt with in this thesis. Both enzymic and chemical conversions, without the use of non-volatile toxic solvents, have been taken into consideration. In addition, isolation of a surfactant from the reaction mixture has been considered to be an integral part of the synthesis.

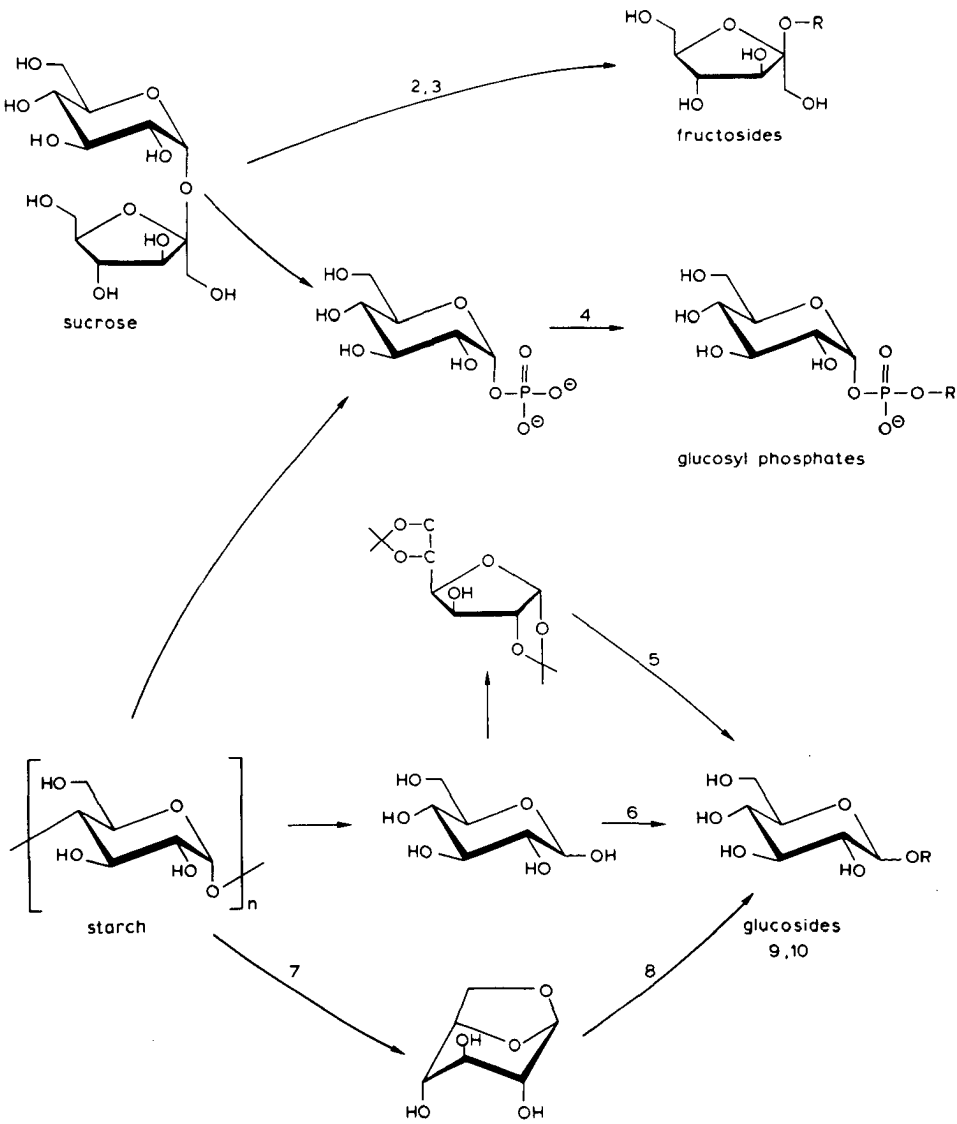
Alkyl glycosides may be prepared selectively using glycosylases as enzymic catalysts. In this respect, the scope and limitations of invertase as an inexpensive glycosylase have been investigated. Chapter 2 deals with the action of invertase in concentrated solutions of sucrose, whereas in Chapter 3 the activity and selectivity of invertase for the preparation of alkyl β-D-fructofuranosides in mixtures of water, alcohol, and cosolvents have been determined.

Chapter 4 describes the esterification of glucose 1-phosphate, which, in principle, leads to anionic surfactants. Glucose 1-phosphate can be conveniently obtained from either sucrose or starch using a phosphorylase.

In Chapter 5 the mechanism of the acid-catalysed butanolysis of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose is outlined. This compound, easily accessible from D-glucose, is well-soluble in fatty alcohols and can

be directly converted to octyl, decyl, and dodecyl α-D-glucopyranosides. In Chapter 6 it is shown that the same reaction can also be successfully applied to D-glucose itself.

Chapter 7 deals with the application of microwave technology to the



Scheme 4. Survey of the reactions dealt with in this thesis. The figures refer to the Chapters.

preparation of 1,6-anhydroglucose from starch and other (1→4)-glucans. The subsequent acid-catalysed alcoholysis of 1,6-anhydroglucose is described in Chapter 8, in particular with respect to kinetic and mechanistic aspects that are of importance for possible selective alkyl D-glucopyranoside formation.

Chapter 9 deals with the crystal structure of anhydrous octyl α-D-glucopyranoside, which is compared to the hemihydrate and monohydrate structures. These compounds possess liquid crystal properties. Finally, in Chapter 10 the solid state and solution properties of octyl α- and β-D-glucopyranoside are discussed.

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CHAPTER 2

INVERTASE-CATALYSED FRUCTOSYL TRANSFER IN CONCENTRATED SOLUTIONS OF SUCROSE*

2.1 INTRODUCTION

Invertase (β-D-fructofuranosidase, EC 3.2.1.26) was used by Michaelis and Menten[1] in their classic kinetic study on the enzymic hydrolysis of sucrose. Interest in the action of invertase in concentrated solutions has been aroused by the commercial production of invert sugar at concentrations up to 3 M sucrose, using invertase from yeast (*Saccharomyces cerevisiae*). At concentrations exceeding 0.2 M, invertase activity was shown to deviate from Michaelis-Menten kinetics since the rate of hydrolysis gradually decreased. This effect has been attributed to the decrease of the concentration of water[2-4], to substrate inhibition[3-5], and substrate aggregation[5,6]. It does not depend on the viscosity of the medium[3,5,7].

A factor which has been neglected in this context is the transfructosylating capability of invertase[8-10]. In the presence of primary alcohols, transfer of β-D-fructofuranosyl units to such alcohols competes with transfer to water. During the hydrolysis of sucrose, fructosyl transfer to the primary hydroxyl groups of sucrose yields small proportions of the non-reducing trisaccharides 1-kestose, 6-kestose, and neokestose by fructosyl transfer to HO-1^{f}, HO-6^{f}, and HO-6^{g}, respectively. Also, reducing disaccharides are formed during the reaction by fructosyl transfer to the primary hydroxyl groups of D-glucose and D-fructose. Eventually, all oligosaccharides are hydrolysed to D-glucose and D-fructose.

A quantitative study[10] showed that the formation of oligosaccharides should not be neglected in 0.29 M sucrose. We assumed that the formation of oligosaccharides would be even more pronounced at increased concentrations of sucrose and consequently would decrease the rate of hydrolysis of

* A.J.J. Straathof, A.P.G. Kieboom, and H. van Bekkum, Carbohydr. Res., **146** (1986) 154.

sucrose. Therefore, we have studied the action of invertase using concentrations of sucrose up to 2.34 M.

2.2 RESULTS AND DISCUSSION

HPLC allowed quantitative analysis of mixtures of D-glucose, D-fructose, 6-kestose, inulobiose, and sucrose; the last peak was shown to contain a small proportion of 6-*O*-(β-D-fructofuranosyl)-D-glucose. 1-Kestose and neokestose were not detected, indicating that these trisaccharides were present at a concentration far below that of 6-kestose[8].

All reactions were performed at 25°C in 0.01 M acetate buffer (pH 4.8). Fig. 1 shows a typical example of the course of the conversion. 6-Kestose was formed during the initial stage of the reaction, but inulobiose was not present in significant amounts before 20% of the sucrose had been converted and reached its maximum concentration at ~ 90% conversion of sucrose,

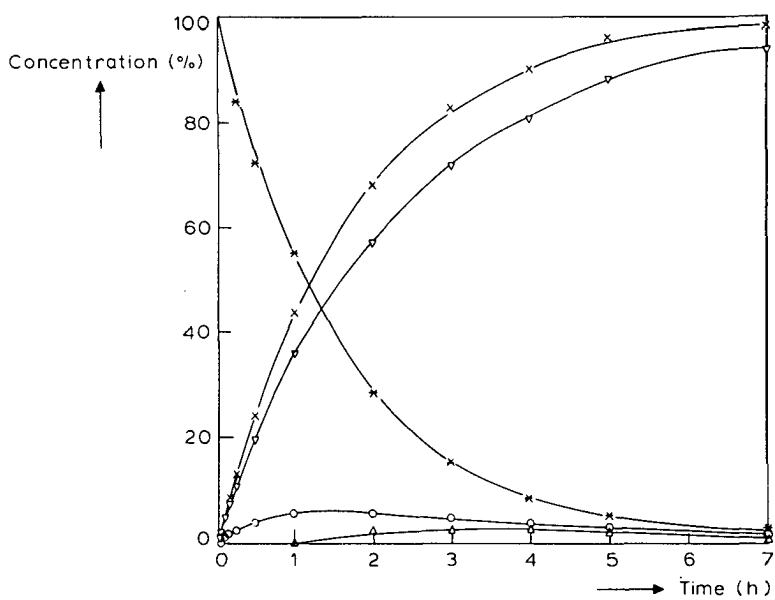

Fig. 1. Conversion of 1.75 M sucrose by invertase (48,000 U/L) in 0.01 M acetate buffer (pH 4.8) at 25°C; ∗, sucrose and 6-O-(β-D-fructofuranosyl)-D-glucose; x, *D-glucose;* ∇, *D-fructose;* o, *6-kestose;* Δ, *inulobiose. Concentrations are relative to the initial molar concentration of sucrose.*

whereas the amount of 6-kestose was at a maximum at ~ 60% conversion, irrespective of the initial concentration of sucrose.

The maximum concentration of these oligosaccharides relative to the initial concentration of sucrose did not increase up to 2.34 M of the latter (Fig. 2). 6-Kestose showed a maximum (7.1 mol%) at 1.3 M sucrose, whereas inulobiose reached its maximum (2.6 mol%) at 1.75 M sucrose.

The observation that higher concentrations of sucrose did not lead to an increase in the concentration of oligosaccharides reflects a change in selectivity of either the formation or hydrolysis of these oligosaccharides relative to the hydrolysis of sucrose. Initial rate studies provided an opportunity for a more detailed investigation of this phenomenon. These rates were therefore determined for concentrations of sucrose in the range 0.02-2.34 M (Fig. 3). The rate of formation of D-fructose was used to calculate the rate of hydrolysis of sucrose. All previous studies concerning invertase kinetics used the rate of formation of D-glucose or reducing sugar, assuming equal concentrations of D-glucose and D-fructose. The present results show that, for concentrated solutions of sucrose, this leads to errors up to 25%, since D-glucose is liberated by both the hydrolysis of sucrose and the formation of kestose.

The initial rate of hydrolysis (r_h), as calculated from the formation of D-fructose, qualitatively shows the behaviour reported before[2-7]. Thus, an increase according to the Michaelis-Menten model up to 0.2 M sucrose was followed by a gradual decrease at higher concentrations. The initial rate of formation of kestose (r_k) showed a much flatter and lower maximum, at ~ 0.8 M sucrose.

The ratio r_k/r_h increased up to 0.3 with increasing concentration of sucrose up to 1.3 M, but decreased at higher concentrations (Fig. 2). This behaviour is in harmony with the pattern of the maximum concentration of 6-kestose, as shown in Fig. 2. It is tempting to ascribe the reduced maximum amount of 6-kestose at concentrations of sucrose exceeding 1.3 M to the decrease in the formation of kestose relative to the hydrolysis of sucrose, and to assume that the ratio of hydrolyses of kestose and sucrose undergoes no pronounced change.

The formation of both 6-kestose and D-fructose will involve one common enzyme-fructosyl complex that can react with sucrose (rate constant k_k) or water (rate constant k_h), respectively[11]. Thus, the ratio of reaction rates (r_k/r_h) is expected to be constant after correction for the molar concentration of sucrose ([S]) and water ([W]) present (equation 1). Fig. 4,

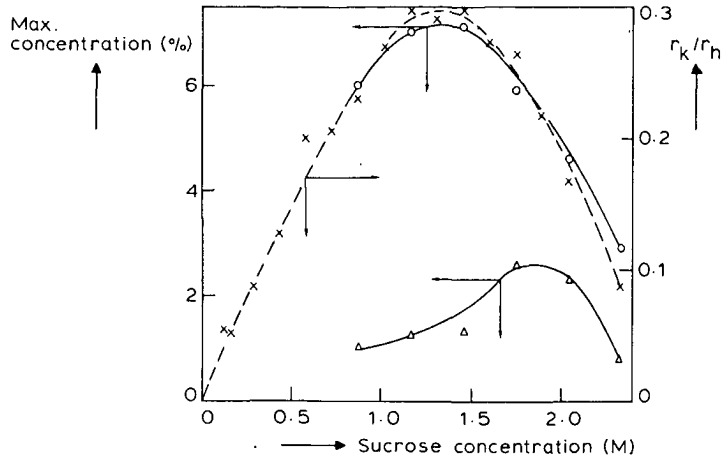

Fig. 2. Left axis: maximum concentration of 6-kestose (o) *and inulobiose* (Δ) *relative to the corresponding initial molar concentration of sucrose. Right axis: ratio* (x) *between the initial rate of formation of kestose and the initial rate of hydrolysis of sucrose.*

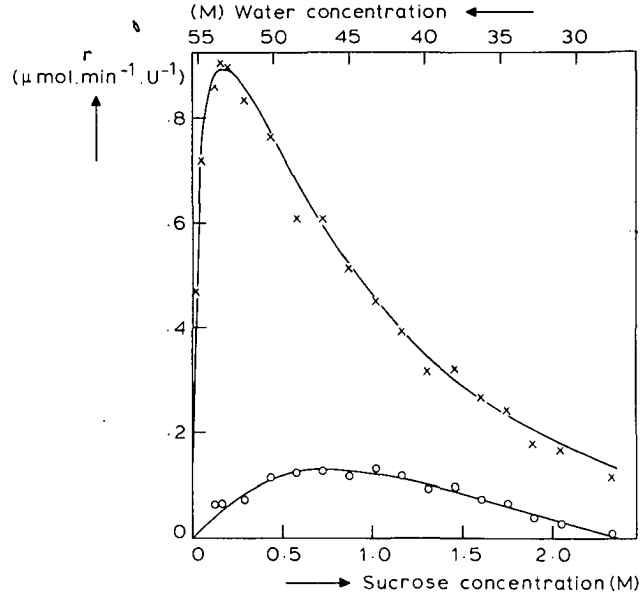

Fig. 3. Initial rates of hydrolysis of sucrose (x) *and formation of kestose* (o) *as a function of the concentrations of sucrose and water. The curves are calculated with the use of equations 4 and 5.*

however, shows that this ratio decreased linearly for concentrations of sucrose exceeding 0.4 M, according to equation 2.

$$\frac{r_k}{r_h} \cdot \frac{[W]}{[S]} = \frac{k_k}{k_h} \qquad (1)$$

$$\frac{r_k}{r_h} \cdot \frac{[W]}{[S]} = 20.6 - 8.86\,[S] \qquad (2)$$

The negative term in equation 2 originates from the non-ideality of concentrated solutions of sucrose. At concentrations up to 2.34 M, the molar ratio of water to sucrose decreases to 12, and intra- and intermolecular hydrogen-bonding of sucrose thus will occur[12-14]. It is assumed that these hydrogen bonds may affect the reactivity of sucrose as a fructosyl acceptor if HO-6^f is involved. The intramolecular hydrogen-bonding, the extent of which is not agreed[12-13], does not seem[13] to involve HO-6^f. Therefore, intermolecular hydrogen-bonding, leading to association of sucrose molecules, is thought to be largely responsible for the reduction in rate. For steric reasons, the HO-6^f groups involved in hydrogen-bonding are considered to become less susceptible to attack by the enzyme-fructosyl complex. Although no quantitative data are available, the extent of this association will be approximately proportional to $[S]^2$. The fraction (α) of sucrose that shows reactivity towards formation of kestose is thus given by equation 3. Substitution of $\alpha[S]$ for $[S]$ in equation 1 fully meets the experimental results of equation 2 for $k_k/k_h = 20.6$ and $c = 0.43\ M^{-1}$.

$$\alpha[S] = [S] - c \cdot [S]^2 \qquad (3)$$

Combes and Monsan[5] introduced the inhibition terms $[S]^2/K_s$ and $[S]^3/K'_s$ in the Michaelis-Menten equation to account for the decrease in the rate of hydrolysis of sucrose at concentrations exceeding 0.2 M. They explained these terms by substrate inhibition of the enzyme-sucrose complex by a second molecule of sucrose and a dimer of sucrose, resulting from intermolecular hydrogen-bonding.

When similar inhibition terms were incorporated in equations 4 and 5, there was good correlation with the experimental results for K_m 37.8 mM, K_i 1.2 M, K'_i 3.3 M^2, k_k 4.7×10^{-7} $min^{-1}.U^{-1}$, and k_h 2.3×10^{-8} $min^{-1}.U^{-1}$ (see Fig. 3).

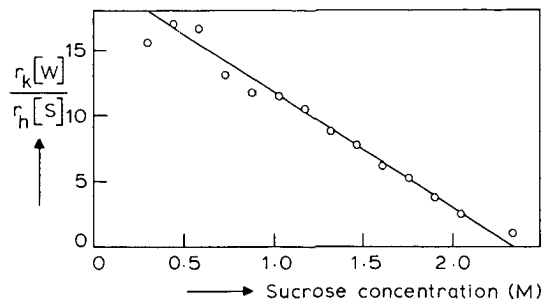

Fig. 4. Ratio between the initial rates of formation of kestose and hydrolysis of sucrose corrected for the concentrations of sucrose and water, as a function of the concentration of sucrose (equation 2).

$$r_k = \frac{k_k \cdot \alpha[S] \cdot [S]}{K_m + [S] + [S]^2/K_i + [S]^3/K_i'} \qquad (4)$$

$$r_h = \frac{k_h \cdot [W] \cdot [S]}{K_m + [S] + [S]^2/K_i + [S]^3/K_i'} \qquad (5)$$

More information about substrate inhibition and hydrogen-bonding in concentrated solutions of sucrose is required for a more detailed understanding of the phenomena.

2.3 EXPERIMENTAL

Invertase was a kind gift of Gist-brocades (Delft, The Netherlands). The specific activity was 240 U (μmol fructosyl units transferred per min per mg) with 0.2 M sucrose.

The conversion of sucrose was started by addition of a buffer solution (5 mL, 0.08 M sodium acetate, pH 4.8) of invertase to aqueous sucrose (35 mL) at 25°C, yielding an acetate buffer (0.01 M, 40 mL) of 720-120,000 U/L of invertase and 0.02-2.34 M sucrose. Samples (2 mL) were added to aqueous silver nitrate to stop the reaction (invertase was inactive in 0.01 M silver nitrate at 25°C) and ethylene glycol was added as internal standard for HPLC.

The HPLC system used has been described in detail elsewhere[15]. The Aminex HPX 87 C column (Ca^{2+} form) at 60°C was eluted with water at 0.6 mL/min. Base-line separation of seven components was achieved. Retention times (min): *A* 6.10, *B* 7.47, sucrose 8.93, glucose 11.2, *C* 13.2, fructose 15.6, ethylene glycol (internal standard) 19.2. Fractions corresponding to these peaks were collected for identification.

TLC[16] was performed on silica gel 60 F254 (Merck) using water-saturated butanol-ethanol (100:40) and detection with aniline-diphenylamine-acetone-85% phosphoric acid (4 mL : 4 g : 200 mL : 30 mL), at 100°C for 10 min.

The amount of *A* was too small for identification. *B* was identified as 6-kestose [β-D-Fru*f*-(2→6)-β-D-Fru*f*-(2↔1)-α-D-Glc*p*] since its eluate yielded D-glucose and D-fructose in the ratio 1:2 on incubation with invertase. In addition, TLC showed a spot, R_{Suc} 0.38, corresponding to 6-kestose [R_{Suc} 0.41[16], *cf.* 1-kestose (0.51) and neokestose (0.75)[16]]. The blue colour of this spot indicated the absence of a reducing fructosyl unit. *C* was assigned to 1-*O*-(β-D-fructofuranosyl)-D-fructose (inulobiose) since the mixture of oligofructosides obtained by partial acid hydrolysis of inulin[17] showed the same peak in HPLC and the same red spot at R_{Suc} 0.68 in TLC. Moreover, increased formation of *C* was observed upon addition of D-fructose to the inverting solution of sucrose. The HPLC eluates of D-glucose and D-fructose showed no additional spots, but sucrose contained a contaminant, R_{Suc} 0.60. The blue colour of this small spot and its increased formation on the addition of D-glucose to the inverting solution of sucrose indicated it to be 6-*O*-(β-D-fructofuranosyl)-D-glucose.

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CHAPTER 3

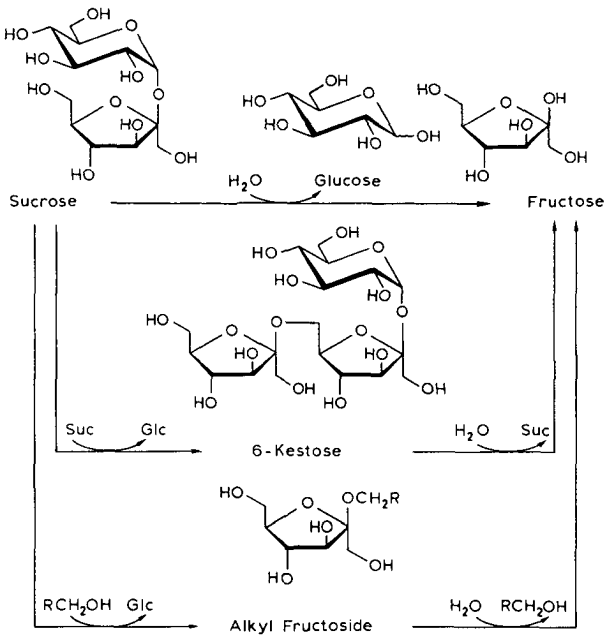
ENZYMIC FORMATION OF β-D-FRUCTOFURANOSIDES FROM SUCROSE: ACTIVITY AND SELECTIVITY OF INVERTASE IN MIXTURES OF WATER AND ALCOHOL*

3.1 INTRODUCTION

The utilisation of protecting and activating groups allows the selective formation of numerous types of glycosidic bonds. The reaction of unprotected carbohydrates with alcohols, however, in the presence of glycosyl transferases (EC 2.4) or glycoside hydrolases (EC 3.2) is much more straightforward. These two classes of enzymes have been called collectively glycosylases by Hehre *et al.*[1], on account of the similar reaction mechanism of alcoholysis and hydrolysis, and the occurrence of many enzymes showing the characteristics of both classes. For instance, invertase (β-D-fructofuranosidase, EC 3.2.1.26) from yeast (*Saccharomyces cerevisiae*), which catalyses the hydrolysis of β-D-fructofuranosides, is also capable of catalysing alcoholysis with primary alcohols[2,3]. Thus, by converting sucrose in water - alcohol mixtures in the presence of invertase, hydrolysis and alcoholysis are parallel reactions and several alkyl β-D-fructofuranosides (up to octyl) have been prepared[4-6]. Since fructosyl transfer also occurs to the primary alcohol groups of sucrose, D-fructose, and D-glucose, small amounts of oligosaccharides may be formed in addition[2]. The trisaccharide 6-kestose, which is formed by fructosyl transfer to HO-6^{f} of sucrose, is the main transfructosylation product in concentrated solutions of sucrose[7]. In the presence of water the fructosides formed will be subject to hydrolysis. The invertase-catalysed reactions of sucrose in mixtures of water and primary alcohol are summarised in Scheme 1.

Optimisation of the preparation of fructosides from sucrose requires

* A.J.J. Straathof, J.P. Vrijenhoef, E.P.A.T. Sprangers, H. van Bekkum, and A.P.G. Kieboom, J. Carbohydr. Chem., in press.



Scheme 1. Invertase-catalysed reactions in solutions of sucrose in aqueous primary alcohol.

knowledge of the effect of high concentrations of alcohols on the selectivity of the reaction, *i.e.* the rates of hydrolysis and alcoholysis. As an extension of our study[7] on the kinetics of invertase-catalysed reactions in concentrated solutions of sucrose, we have investigated the scope and limitations of the use of invertase for the preparation of alkyl β-D-fructofuranosides in mixtures of water and organic solvents (including reactive primary alcohols and non-reactive solvents). Also some experiments have been performed in anhydrous solvents.

3.2 RESULTS AND DISCUSSION

Invertase activity in anhydrous solvents

The activity of invertase in the absence of water was tested using both lyophilised and phosphorous pentoxide-dried invertase at 25 and 45°C (ref. 8, *cf.* ref. 9-10). No conversion of sucrose was detected in suspensions in

anhydrous 1-butanol, 1-octanol, or mixtures thereof as the medium. The lack of conversion of sucrose might partly be due to the low solubility of sucrose in these alcohols (*e.g.* the solubility of sucrose in butanol at 80°C is 0.12 g/100 g[11]). Upon addition of 10 volumes of water the formulations in 1-octanol and 1-butanol showed complete conversion of sucrose. Thus, no complete irreversible inactivation of invertase occurs in 1-octanol or 1-butanol. In anhydrous pyridine, which is a much better solvent for sucrose, no sucrose conversion was detected in the presence of 5% 1-butanol. Upon addition of 10 volumes of water to the pyridine suspension sucrose was hydrolysed at a very low rate. The basic pyridine will affect the state of protonation of the active site of invertase, thus decreasing its activity. A sharp decrease in invertase activity was also observed upon addition of 10% pyridine to aqueous sucrose.

Invertase activity in water - organic solvent mixtures

Since no formation of fructosides was observed under essentially anhydrous conditions, we have studied the activity of invertase in mixtures of water and organic solvents up to compositions allowing complete dissolution of 0.1 M sucrose at 25°C. In the case of reactive primary alcohols (Fig. 1A) the conversion rate decreases with increasing concentration of

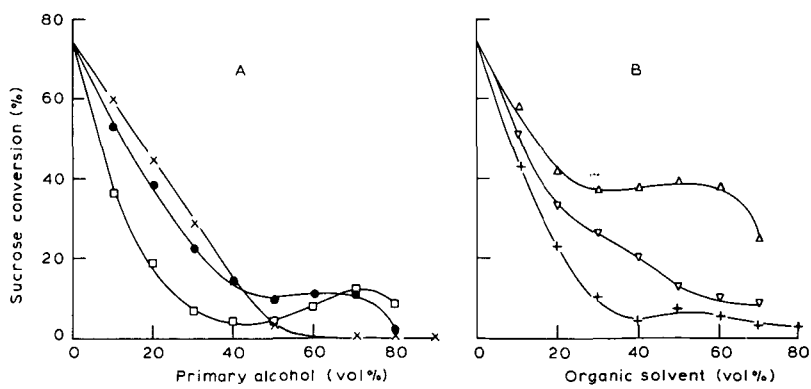
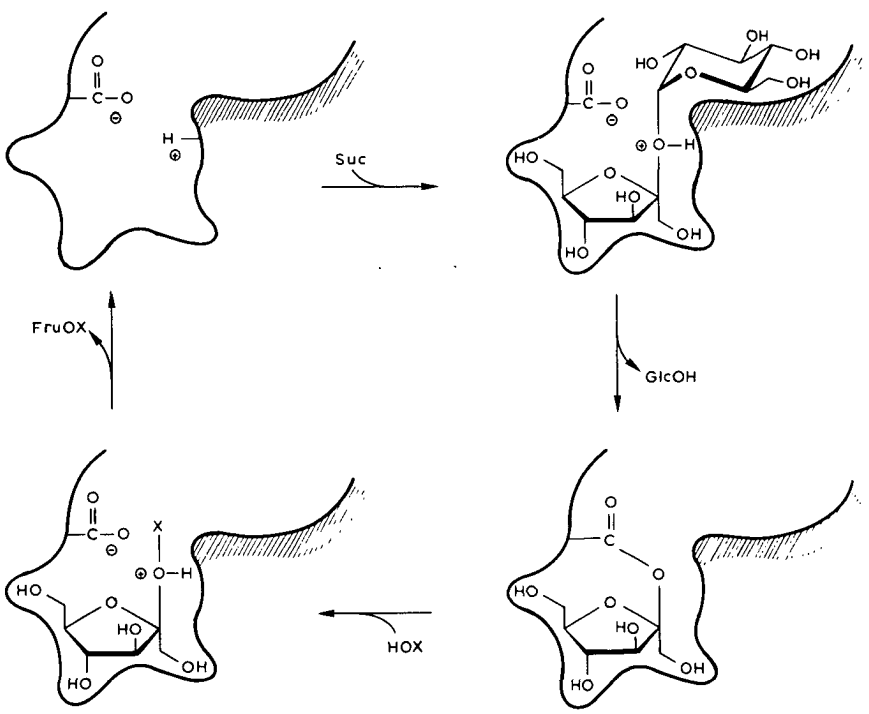


Fig. 1. Sucrose conversion by invertase after 5 min reaction (0.1 M sucrose, 100 mg/L invertase, pH 4.8, 25°C). A. In mixtures of water and methanol (x), *ethanol* (●), *or allyl alcohol* (□). *B. In mixtures of water and tert-butanol* (Δ), *acetone* (∇), *or 1,4-dioxane* (+).

alcohol, with a relative maximum at high concentrations of ethanol or allyl alcohol. A related behaviour was observed for the non-reactive cosolvents dioxane and *tert*-butanol (Fig. 1B). Thus, the maxima do not originate from alcoholysis occurring at high concentration of primary alcohol and indicate the complex nature of the effect of organic solvents on the activity of invertase. Up to 70% v/v organic solvent (50% for methanol), invertase activity is still sufficient for preparative purposes.

Active site and mode of action of invertase

Invertase is a glycoprotein with a polysaccharide content exceeding 50%. The primary structures of the polysaccharide branches[12] and the protein backbone[13] have been elucidated, but the structure of the active site of invertase is not known. Some important information has been obtained from hydrolysis studies (*cf.* Scheme 2). No β-D-fructofuranoside is known that is



Scheme 2. Model of the active site of invertase during hydrolysis (HOX = water) or alcoholysis (HOX = primary alcohol) of sucrose. The non-polar part of the aglycon site is shaded.

not hydrolysed by invertase. Upon minor modification of the β-D-fructofuranosyl moiety, however, hydrolysis has never been observed[14-17]. Thus, invertase has a very specific fructosyl binding site and a rather unspecific aglycon binding site. The aglycon site seems to have a somewhat non-polar character, since we observed that butyl β-D-fructofuranoside (K_m = 9.4 mM) showed a higher affinity for invertase than sucrose (K_m = 38 mM)[18]. The non-polar character of the aglycon site is in accordance with the weak binding of raffinose (K_m = 240 mM)[19] and the very weak inhibition by D-glucose (partial non-competitive inhibition, K_I = 410 mM)[18].

The catalytic site of invertase is assumed to contain an imidazolium and a carboxylate group[21]. Upon protonation of the glycosidic oxygen atom of sucrose by the imidazolium group, cleavage of the bond between this atom and fructosyl C-2 occurs. On the analogy of the mechanistically related enzyme levansucrase[20], an ester bond between C-2 and the carboxylate group of the enzyme will be formed. Formation of a β-D-fructofuranosyl ester is most probable because inversion at fructosyl C-2 is sterically unfavourable[23]. α-D-Glucopyranose is liberated from the active site and the cleavage of the ester bond by water or a primary alcohol yields β-D-fructofuranose[24] or alkyl β-D-fructofuranoside, respectively. All reactions thus are supposed to proceed with retention of configuration (Scheme 2).

Invertase selectivity in water - alcohol mixtures

The aglycon site of invertase will show hydrophobic interactions with aliphatic alcohols, without much specificity towards the shape of the molecule. Sucrose, that has been shown to be a weak substrate inhibitor[7,20], will probably bind in a rather nonspecific manner at the aglycon site of invertase or the invertase-fructosyl complex. Thus, hydrolysis was retarded[20], but sucrolysis was retarded even more[7]. Non-reactive binding of sucrose close to the aglycon site (Scheme 3, I and II) at high sucrose concentration can explain these results. According to the substrate inhibition observed, the reaction I→II is slower than IV→V.

The nature of these inhibition phenomena might be established by studying the action of invertase in the presence of alcohols that form more specific complexes at the aglycon site. Thus, the relative importance of hydrolysis, alcoholysis, and sucrolysis has been studied in 1 M aqueous *tert*-butanol and in 1.5 M aqueous allyl alcohol (10% v/v in both cases) at variable concentration of sucrose. Use has been made of initial rates in order to eliminate any effect of product inhibition and hydrolysis of newly

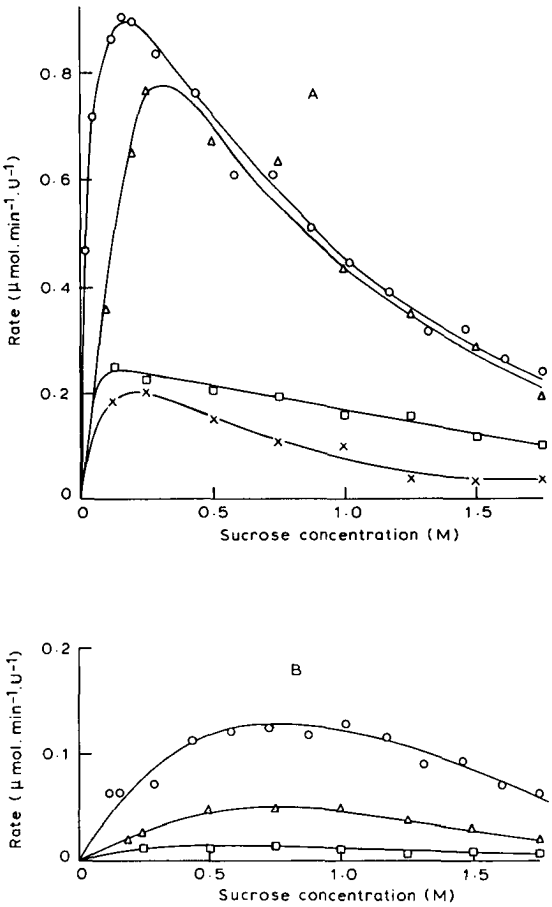
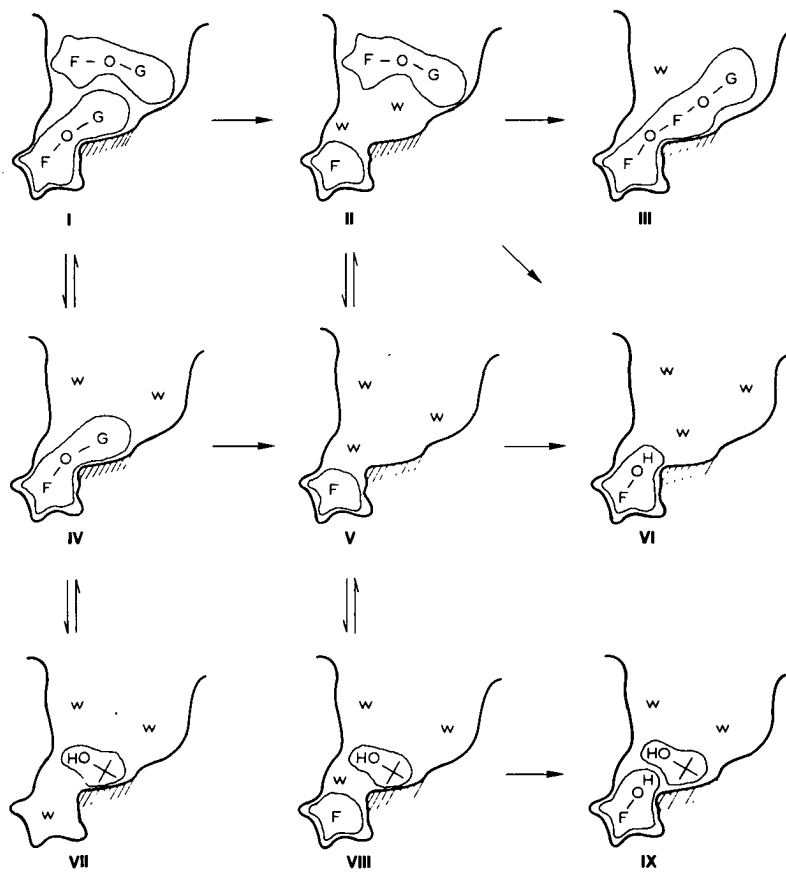


Fig. 2. Initial rates of hydrolysis and alcoholysis of sucrose (pH 4.8, 25°C). A. Hydrolysis in water (o) *and in 10% tert-butanol* (Δ)*; Hydrolysis* (□) *and allylolysis* (x) *in 10% allyl alcohol. B. Sucrolysis in water* (o)*, 10% tert-butanol* (Δ)*, and in 10% allyl alcohol* (□).

formed fructosides. In Fig. 2 these rates are compared to the initial rates in the absence of organic solvents[7].

tert-Butanol only decreases the rate of hydrolysis if [Sucrose] < 0.4 M, which is due to a higher apparent K_m of sucrose in 10% *tert*-butanol (K_m = ~ 270 mM) than in water (K_m = 38 mM). This means that *tert*-butanol behaves as a weak competitive inhibitor (K_I = ~ 170 mM) because of complexation at the non-polar part of the aglycon site of invertase (Scheme 3, VII). Sucrose

will form a stronger complex (IV) because of its affinity to the fructosyl site, but after liberation of glucose (IV→V) complexation of *tert*-butanol at the aglycon site of the enzyme-fructosyl complex will occur (V→VIII) without much competition by sucrose. This complexation hardly affects the reaction rate of the enzyme-fructosyl complex with water, according to the similar shapes of the curves of water and 10% *tert*-butanol at [Sucrose] > 0.4 M in Fig. 2A. Thus, the rate of VIII→IX is comparable to the rate of V→VI. According to the corresponding curves in Fig. 2B, however, the reaction rate of the enzyme-substrate complex V with the large sucrose molecule is reduced



Scheme 3. Effect of sucrose (G-O-F), *tert-butanol* (+OH), *and water* (w) *on the formation of fructose* (F-OH) *and 6-kestose* (F-O-F-O-G) *in the active site of invertase. The non-polar part of the aglycon site is shaded.*

by a factor 3 in the presence of *tert*-butanol. Formation of VIII inhibits the formation of II with subsequent reaction to III. *tert*-Butanol seems to block the catalytic site for the large sucrose molecule, whereas the entrance of water to the catalytic site is not inhibited.

Allyl alcohol will bind at the aglycon site in a manner comparable to *tert*-butanol in complex VII, but proves to be a more efficient inhibitor. Binding of allyl alcohol to the enzyme-fructosyl complex will result in allyl fructoside formation, but additional inhibition may occur by reverse orientation of the allyl alcohol molecule at the non-polar site (*i.e.* its

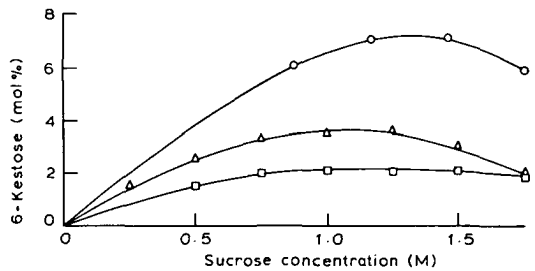


Fig. 3. Maximum concentration of 6-kestose relative to the corresponding initial concentration of sucrose in the absence of organic solvent (o), *in 10% tert-butanol* (Δ), *and in 10% allyl alcohol* (□) *(pH 4.8, 25°C).*

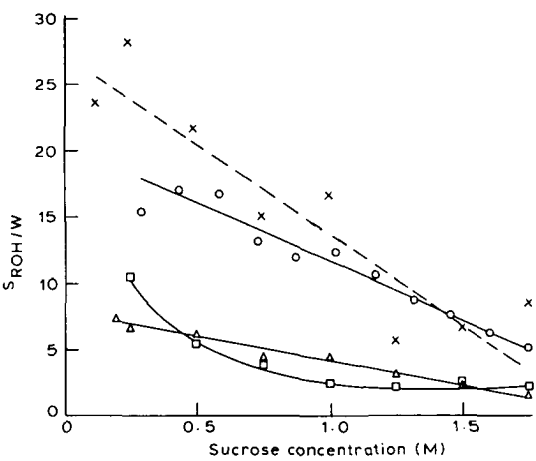


Fig. 4. Selectivity towards sucrolysis in the absence of organic solvent (o), *in 10% tert-butanol* (Δ), *and in 10% allyl alcohol* (□); *selectivity towards allylolysis in 10% allyl alcohol* (x).

hydroxyl group pointing away from the fructosyl group).

The inhibiting effect of *tert*-butanol and allyl alcohol on the sucrolysis as determined by these initial rate studies, is reflected by the maximum amount of 6-kestose observed in the course of the reaction (Fig. 3).

A comparison of the effect of various alcohols on the different reaction rates can be made after correction of the ratio of the initial rate of alcoholysis (r_a) to hydrolysis (r_h) for the molar ratio of water to alcohol ([W]/[ROH]). The molar selectivity towards alcoholysis ($S_{ROH/W}$) thus defined is a measure of the reactivity of sucrose towards primary alcohols relative to water, in the presence of invertase (equation 1).

$$S_{ROH/W} = \frac{r_a}{r_h} \cdot \frac{[W]}{[ROH]} \qquad (1)$$

Fig. 4 shows that $S_{ROH/W}$ has not a fixed value for either sucrose or allyl alcohol, but it is clear that these alcohols are better fructosyl acceptors than water ($S_{ROH/W} > 1$). Although additional effects, like variation in water, sucrose, and alcohol activities[25] and differences in solvent composition in the microdomain of invertase relative to the bulk solvent[26-27] will be of importance as well, the decrease in $S_{ROH/W}$ observed upon addition of sucrose, allyl alcohol, or *tert*-butanol is assumed to be largely the result of interactions in the enzyme cavity according to the picture given above.

With increasing concentration of aliphatic alcohol, saturation of the aglycon site will occur, and the ratio of reaction rates of alcoholysis and hydrolysis will show only a small increase. The selectivity, which is calculated after correction of the concentrations, will decrease. Thus, the selectivity of ethanolysis $S_{EtOH/W}$ in 0.44 M sucrose in the presence of 10, 30, 50, and 70% aqueous ethanol was 7, 3, 2, and 0.5, respectively. As a consequence, the maximum amount of ethyl fructoside formed was 9, 9, 13, and 10%, respectively. Thus, the decreasing relative molar selectivity towards alcoholysis opposes the more favourable molar ratio of alcohol to water.

This result applies to all alcoholysis reactions studied by us. Optimal formation of fructosides occurs therefore at relatively low concentration of sucrose (Fig. 4) together with intermediate concentrations of

alcohol (40-75% v/v, Fig. 5). In general, the maximal amount of alkyl β-D-fructofuranoside observed decreases with increasing size of the fructosyl acceptor.

The present work and interpretation may be of relevance for the enzymic preparation of α-[28] and β-[28-29]galactosides, α-[30] and β-[29-31]glucosides, α-mannosides[28], and α-maltosides[32] by alcoholysis of glycosides, which have also been performed at a remarkably low concentration of alcohol (~ 10-60% v/v). This low alcohol content will probably have favoured the solubility of the substrates and the stability of the enzymes applied. Initial reaction rates of alcoholysis and hydrolysis, which might reveal some general negative effect of high alcohol concentration on the selectivity of glycosylases towards alcoholysis, were, however, not reported.

The enzymic formation of alkyl fructofuranosides (this work) appears to be less favourable than that of alkyl aldopyranosides[28-31]. This is due to

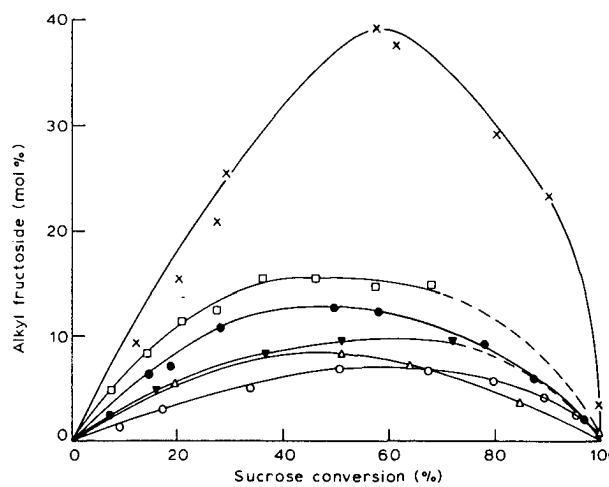


Fig. 5. Course of the alkyl β-D-fructofuranoside concentration (relative to the initial sucrose concentration) at the optimum conditions observed for fructoside formation in the presence of invertase at 25°C, pH 4.8: x, *methyl fructoside (40% v/v MeOH, 150 g/L sucrose);* ●, *ethyl fructoside (50% v/v EtOH, 150 g/L sucrose);* ▼, *propyl fructoside (55% v/v PrOH, 150 g/L sucrose);* Δ, *butyl fructoside (50% v/v BuOH, 100 g/L sucrose, heterogeneous);* □, *allyl fructoside (75% v/v AllOH, 100 g/L sucrose);* o, *6-kestose (500 g/L sucrose).*

both kinetic and thermodynamic reasons. The steric hindrance for alcohols (relative to water) to react with an enzyme-fructofuranosyl complex will be much larger than with *e.g.* an enzyme-glucopyranosyl complex, because of the bulky fructosyl 1-CH_2OH group. Therefore, secondary and tertiary alcohols do not react with the fructosyl-invertase complex, whereas their enzymic conversion into alkyl aldopyranosides is well-known[28-31]. Furthermore, it must be noted that differences in hydrophobic nature and in bulkiness of the alcohols might strongly influence their ability to bind at the aglycon site and, consequently, their relative apparent reactivity. In addition, fructofuranosides are in aqueous solution thermodynamically less stable than glucopyranosides[33]. Thus, in concentrated aqueous solutions of D-glucose, enzymic formation of disaccharides by reversion reactions occurs[34], but equilibration of concentrated aqueous solutions of D-fructose (or invert sugar) in the presence of invertase does not show any disaccharide formation.

3.3 EXPERIMENTAL

Reaction procedure

Sample preparation was essentially the same as reported before[7]. A buffer solution (5 mL, 0.08 M sodium acetate, pH 4.8) of invertase (Maxinvert powder, 240 U/mg[7], Gist-brocades, Delft) was added to a solution (35 mL) of sucrose in aqueous alcohol at 25°C, yielding a reaction mixture of the correct composition. Samples were added to aqueous silver nitrate. Ethylene glycol or D-glucitol was added as internal standard for HPLC and the solvent was evaporated as far as required. Hydrolysis of butyl β-D-fructofuranoside was performed on a 4 mL scale starting with 20, 50, and 100 mM aqueous solutions of this compound. For initial rate determinations five samples at < 10% conversion were analysed.

HPLC analysis

HPLC of alkyl fructosides was performed using a Waters Assoc. M45-pump, a cartridge packed with 3-aminopropyl-triethoxysilane-modified silica contained in a Waters Assoc. RCM 100 module, and a Waters Assoc. R401 differential refractometer. The flow of acetonitrile-water (85:15) was 1 mL/min at 25°C. Retention times (min): Alkyl β-D-fructofuranosides: butyl 5.12, propyl 5.90, ethyl 6.36, methyl 7.98; D-fructose 10.6, D-glucose 12.5, sucrose

22.1. More reproducible results were obtained using ion-moderated partitioning chromatography. An Aminex HPX 87C column at 60°C was eluted with water at 0.6 mL/min[7,35]. The alkyl β-D-fructofuranosides, however, were not base-line separated from glucose or fructose. Allyl β-D-fructofuranoside had the same retention time as D-glucose and was therefore determined by a molar balance: [AllFru] = [Suc] - [Fru] - 2[6-kestose]. HPLC peaks of alkyl β-D-fructofuranosides were assigned upon comparing the chromatograms of anomeric mixtures of alkyl D-fructosides (prepared according to the Fischer-method[36]) before and after incubation with invertase in aqueous solution.

Butyl β-D-fructofuranoside

A solution of sucrose (60 g) in acetate buffer (200 mL, 0.02 M, pH 4.8) and 1-butanol (200 mL) was incubated with invertase (200 mg) at 25°C during 15 min. Sodium carbonate was added up to pH 10 and the solvents were removed *in vacuo*. The syrup thus obtained was extracted with boiling ethanol. Ethanol was removed *in vacuo* and the extraction was repeated until a syrup (6.6 g) was obtained which was enriched in butyl β-D-fructofuranoside. Chromatography over Silica Gel 60 (300 cm^3, Merck) with ethyl acetate (150 mL), ethanol-ethyl acetate 1:9 (300 mL), and 1:4 (200 mL), and collection of the fractions between 400 and 640 mL, yielded 0.80 g butyl β-D-fructofuranoside as a syrup, which was pure according to HPLC, and was completely hydrolysed to fructose and butanol upon incubation with invertase.

Test of invertase activity under anhydrous conditions

Anhydrous invertase was prepared by lyophilisation from 0.5 M sodium citrate buffer (pH 4.7) or by drying to constant weight at 15 mm Hg in the presence of an excess of either zeolite NaA or phosphorous pentoxide. Microcrystalline sucrose (0.25 g, particle size < 100 μm, dried on phosphorous pentoxide) was suspended in 1-octanol, 1-butanol, or pyridine (5 mL, dried on zeolite NaA). 1-Butanol (0.25 mL), containing anhydrous sodium acetate (0.1 M) and acetic acid (0.1 M), was added. After addition of dried invertase (0.1 g) the suspension was stirred vigorously for 24 h. TLC was performed on silica gel 60 F254 (Merck) with chloroform-methanol-water (60:35:6) and detection with sodium metaperiodate-tolidine[37]. The limit of detection was < 1% sucrose conversion.

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double inversion of configuration has only been obtained for sucrose phosphorylase. Possible double inversion for invertase, *i.e.* an α-D-fructofuranosyl-enzyme intermediate, does not influence the further mechanistic considerations.

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CHAPTER 4

A NOTE ON THE ESTERIFICATION OF α-D-GLUCOPYRANOSYL PHOSPHATE WITH BENZYL HALIDES*

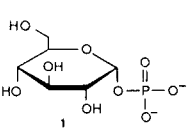
4.1 INTRODUCTION

α-D-Glucopyranosyl phosphate (1) is readily accessible by enzymic conversion of sucrose[1] or starch[2] with inorganic phosphate in the presence of a phosphorylase. The use of 1 as a starting material in organic synthesis, however, has been rather limited so far. An example[3-5] involves esterification of the phosphate group in a three-step synthesis: (*i*) acetylation of the pyridinium salt of 1 to its tetra-*O*-acetyl derivative, (*ii*) reaction with an alcohol in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride, and (*iii*) deacetylation.

In the context of our search to surfactants based on carbohydrates, we are interested in a one-step conversion of 1 into long-chain phosphoric acid esters. Either fatty alcohol or alkylbenzene derivatives are considered as suitable ester-forming reagents.

It is known[6-9] that non-carbohydrate phosphoric acid triesters can be obtained by reaction of tetramethylammonium salts of mono- or dialkyl phosphates in the presence of alkyl halides. Bauman[9] was able to prepare diesters by addition of one equivalent of an alkyl halide to monoalkyl phosphates in acetonitrile.

In order to develop a similar direct esterification procedure for sugar



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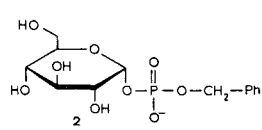

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* A.J.J. Straathof, A.P.G. Kieboom, and H. van Bekkum, Recl. Trav. Chim. Pays-Bas, 104 (1985) 65.

phosphates, we have studied the conversion of the bis(tetrabutylammonium) salt of 1 with benzyl halides as model alkylating compounds. It was expected that the phosphate group of 1 would show enhanced reactivity towards benzyl halides in the absence of protic solvents, since no strong ion-pair can be formed with the tetrabutylammonium cation. Moreover, the tetrabutylammonium cation was chosen for solubility reasons.

4.2 EXPERIMENTAL

Analytical methods

^1H NMR spectra were recorded on a Varian T-60 spectrometer at 60 MHz. Chemical shifts are reported in ppm relative to internal tetramethylsilane (or sodium 3-(trimethylsilyl)-1-propanesulfonate in case of D_2O as solvent). ^{31}P NMR spectra were recorded on a Nicolet NT-200 WB spectrometer at 80.99 MHz with 1% phosphoric acid in D_2O as external standard. HPLC analyses were performed using a reverse phase column (Nucleosil C-18, Waters Assoc.) at 20°C. Mobile phases were 0.01 M tetrabutylammonium hydroxide in 25% aqueous methanol, buffered at pH 6.25 with 85% phosphoric acid (eluent A), and 0.005 M tetrabutylammonium hydroxide in 60% aqueous methanol, buffered at pH 7.3 with 85% phosphoric acid (eluent B). A Rheodyne 7125 injector was used and eluents were pumped at 1.0 mL/min by a Waters Assoc. M6000A pump. For detection, a differential refractometer (Waters Assoc. R401) and an UV spectrophotometer (Pye Unicam LC3, 260 nm) were used. Typical HPLC chromatograms for both eluents are shown in Fig. 1.

Bis-(tetrabutylammonium salt of α-D-glucopyranosyl phosphate (1)

(*i*) The dipotassium salt of 1 was converted into the barium salt by standard procedures[10]. The barium salt trihydrate of 1 (4.495 g, 10.0 mmol) was dissolved in water (100 mL) and added to a solution of bis(tetrabutylammonium) sulfate (10.0 mmol), prepared from tetrabutylammonium hydroxide (12.97 g of a 40% aqueous solution, 20.0 mmol) and sulfuric acid (50 mL 0.2 M, 10.0 mmol). The precipitated barium sulfate was removed by filtration through Hyflo. The filtrate was concentrated to a hygroscopic syrup at 0.5 mm Hg and dissolved in acetonitrile. The solution was dried with zeolite Na-A, and adjusted to a volume of 50.0 mL to obtain a 0.2 M solution of bis(tetrabutylammonium) α-D-glucopyranosyl phosphate.

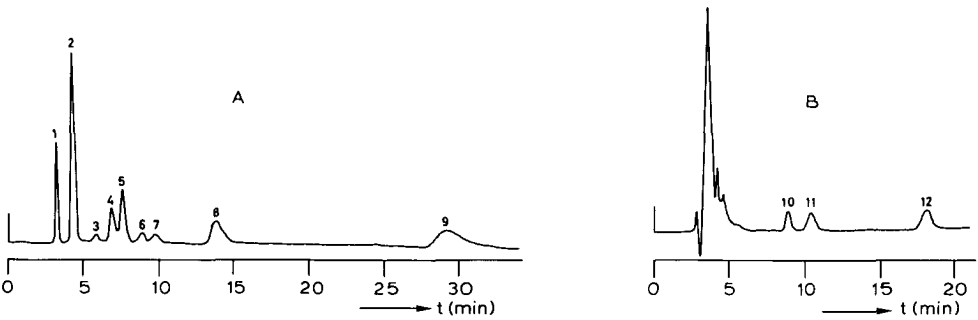


Fig. 1. HPLC chromatograms of reaction mixtures of α-D-glucopyranosyl phosphate (1) with benzyl chloride. Refractive index detection. (A) Eluent A. (1), (4), and (6) are system peaks. The other peaks are: (2) acetonitrile, (3) di-D-glucopyranosyl phosphate, (5) chloride, (7) α-D-glucopyranosyl phosphate, (8) 1-butanol (internal standard), (9) D-glucopyranosyl benzyl phosphate. (11) and (12) are not eluted by eluent A. (B) Eluent B. Peaks (1) to (9) are unresolved, (10) 3-phenyl-1-propanol (internal standard), (11) dibenzyl phosphate, (12) benzyl chloride.

(*ii*) Alternatively, a solution of the dipotassium salt of **1** (745 mg, 2 mmol) in water (10 mL) was eluted over a tenfold excess of cation-exchange resin (AG 50W-X8, 200-400 mesh, BioRad, tetrabutylammonium) with water (20 mL). The combined eluates were concentrated, and after repeated addition and evaporation of acetonitrile, the bis(tetrabutylammonium) salt of **1** was obtained as a syrup. Acetonitrile was added to a volume of 10 mL.

Solutions obtained by either method contained no impurities according to HPLC. ^{1}H NMR (acetonitrile): δ 5.45 (dd, $^3J_{H\text{-}1,P}$ 6.8 Hz, $^3J_{H\text{-}1,H\text{-}2}$ 3.4 Hz).

The bis(tetrabutylammonium) salt of **1** was easily soluble in acetonitrile as well as in methanol, ethanol, *tert*-butanol, acetone, and dichloromethane, but was just slightly soluble in diethyl ether or dioxane.

Reaction of α-D-glucopyranosyl phosphate with benzyl halides

Reactions were carried out by boiling 2-mL aliquots of the 0.2 M solution of the bis(tetrabutylammonium) salt of **1** in acetonitrile under reflux with different amounts of benzyl halides, with exclusion of moisture. Internal standards added to the reaction mixture for HPLC analysis were 1-butanol when using eluent A, or 3-phenyl-1-propanol when using eluent B.

Samples of the reaction mixture were analysed by HPLC or NMR without pretreatment, except for ^{31}P NMR where 20% deuteroacetone was added for internal lock.

α-D-Glucopyranosyl benzyl phosphate (2)

Benzyl chloride (253 mg, 2 mmol) was added to 10 mL 0.2 M bis(tetrabutylammonium) salt of 1 in acetonitrile. The mixture was boiled for 90 min under reflux, concentrated to a syrup and dissolved in water (10 mL). After elution over a tenfold excess of cation-exchange resin (AG 50W-X8, 200-400 mesh, BioRad, Li^+) and washing of the column with water (20 mL), the combined eluates were evaporated to dryness. The solid obtained was extracted several times with acetone to remove lithium chloride. The residue was treated with 20 mL 90% ethanol, unreacted lithium salt of 1 was removed by filtration, and the solvent was evaporated.

The solid obtained (620 mg, 87%) contained no contamination according to HPLC; $[\alpha]_D^{25}$ +86.7° (*c* 1.9 H_2O), m.p. 169°C (dec.). NMR data (D_2O): δ_P -1.07 (m, $^3J_{H-1,P}$ 6.88 Hz, $^3J_{benzyl-H,P}$ 6.88 Hz, $^4J_{H-2,P}$ 2.81 Hz); δ_H 5.60 (dd, $^3J_{H-1,P}$ 6.8 Hz, $^3J_{H-1,H-2}$ 3.2 Hz); 5.05 (d, $^3J_{benzyl-H,P}$ 6.9 Hz).

4.3 RESULTS AND DISCUSSION

Reaction with one equivalent of benzyl chloride

The reaction between equimolar amounts of the bis(tetrabutylammonium) salt of 1 and benzyl chloride in acetonitrile at 82°C yielded 85-90% of the monobenzylated product 2, which was identified by means of NMR spectroscopy. The value of $^3J_{H-1,H-2}$ 3.2 Hz confirmed the α-configuration[11] of the compound obtained[12].

Addition of 1% of water (by volume) to the reaction mixture doubled the time necessary for 85-90% conversion of 1 into 2. This indicated the negative influence of hydration on the reactivity of the phosphate group.

Reaction with two equivalents of benzyl chloride

When a reaction was carried out between the bis(tetrabutylammonium) salt of 1 and two equivalents of benzyl chloride, the amount of 2 decreased after 30 min reaction time as shown in Fig. 2. The decrease of 2 was not accompanied by a decrease in the concentration of benzyl chloride. Clearly,

the excess of benzyl chloride—accelerated in a catalytic fashion the conversion of 2 into other products.

Analysis of the reaction mixture by ^{1}H NMR showed that the double doublet of the anomeric proton at 5.45 ppm disappeared during the decrease in concentration of 2, demonstrating a rupture of the α-glucosylic bond. Simultaneously, a number of new signals appeared in the region of 4.5-5.0 ppm, including a doublet with *J* 7 Hz which probably belongs to a new compound with a benzyl–phosphate bond.

^{31}P NMR showed the formation of several phosphorous containing products, with *J* 6-7 Hz for all signals. In addition to the quartet belonging to 2, another quartet and a quintet were present. Three minor signals, possibly triplets, were visible as well. In conclusion, five new phosphoric acid esters were formed.

HPLC showed that formation of benzyl alcohol was negligible. Dibenzyl phosphate, however, was a major side product, explaining the presence of a

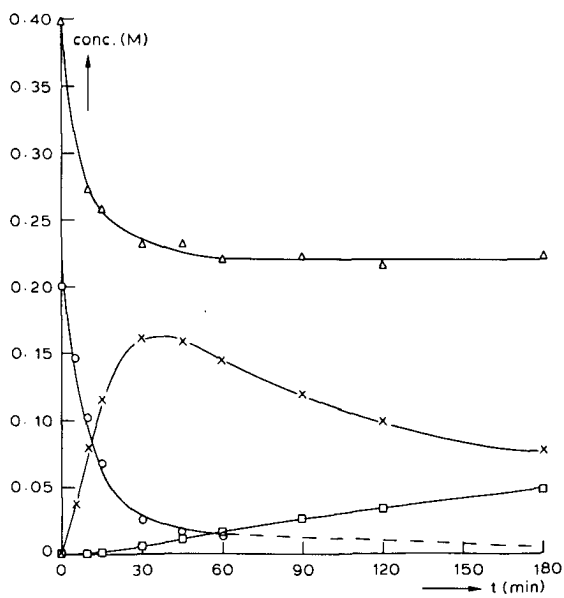
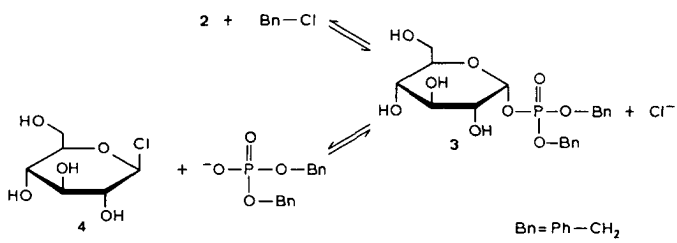


Fig. 2. Conversion of the bis(tetrabutylammonium) salt of α-D-glucopyranosyl phosphate (1) with two equivalents of benzyl chloride in acetonitrile at reflux temperature. o, *1;* ×, *D-glucopyranosyl benzyl phosphate;* Δ, *benzyl chloride;* □, *dibenzyl phosphate.*

quintet in ^{31}P NMR. The HPLC-peak of **2** broadened during the reaction, which is probably due to α,β-anomerisation. In addition, a third side product became visible with HPLC, with retention in between **1** and D-glucose. This product contained no benzyl group as appeared from the absence of UV absorption at 260 nm.

Reaction mechanism

The formation of dibenzyl phosphate and other side products in the presence of an excess of benzyl chloride may be explained by the increased rate of formation of the triester α-D-glucopyranosyl dibenzyl phosphate **3**, which readily reacts with chloride ion to give phosphoric acid diesters and either benzyl chloride or β-D-glucopyranosyl chloride **4** (Scheme 1).



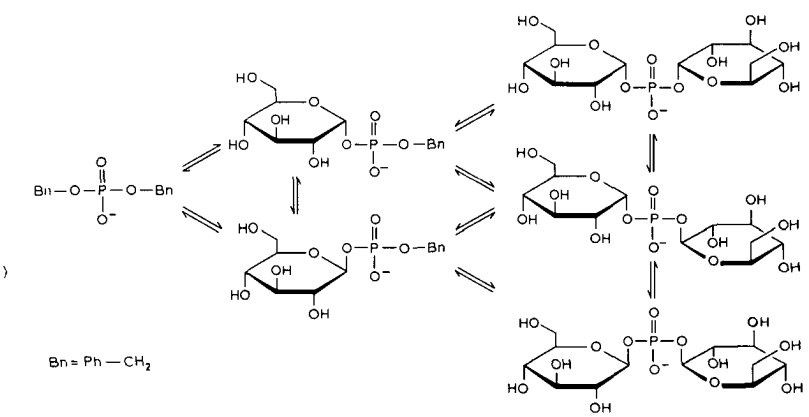
Scheme 1

The instability of phosphoric acid triesters in acetonitrile at reflux temperature in the presence of chloride was confirmed by the rapid decomposition of tribenzyl phosphate into dibenzyl phosphate and benzyl chloride upon addition of tetrabutylammonium chloride.

With respect to the formation of **4**, it may be mentioned that Lemieux and Hayami[13] have found anomerisation of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride by tetraethylammonium chloride under comparable conditions. At 30°C, 93-95% of this compound was converted into its α-anomer after about ten hours equilibration. At 40°C, or with the 3,4,6-tri-*O*-acetyl derivative of **4**, the anomerisation reaction proved to be several times faster[13]. From these data, we estimate that in our case (non-acetylated β-D-glucopyranosyl chloride at 82°C), substantial conversion into the α-anomer occurs within a few minutes.

The mixture of anomeric D-glucosyl chlorides thus formed will produce a mixture a phosphoric acid triesters upon reaction with 2. These, in turn, will react to a mixture of diesters and D-glucosyl chlorides or benzyl chloride.

According to this mechanism, a dynamic equilibrium mixture of phosphoric acid diesters and triesters will be ultimately formed. Supposing no measurable concentrations of triesters to be present because of their relative instability, the only phosphorous containing compounds detectable in the reaction mixture will be six diesters: dibenzyl phosphate, compound 2 and its β-anomer, and three di-D-glucopyranosyl phosphates (α,α'-, α,β'-, and β,β'-, see Scheme 2). This is in agreement with the ^{31}P NMR data, and, assuming that the various anomers are not separated, also with the HPLC data. Since the concentration of the highly reactive D-glucosyl chloride is negligible, no consumption of the excess of benzyl chloride occurs, and the concentrations of dibenzyl phosphate and di-D-glucosyl phosphates remain equal.



Scheme 2. Interconversion of phosphoric acid diesters "catalysed" by benzyl chloride: each interconversion involves (i) attack of the diester by either benzyl or D-glucosyl chloride giving a phosphoric acid triester and chloride; (ii) attack of the intermediate triester by chloride and formation of a new diester and benzyl or D-glucosyl chloride.

The mechanism suggested here may also be responsible for the "dephosphorylation" phenomenon observed by Shibaev *et al.*[14] during the

silylation of 1 with an excess of trimethylsilyl chloride. Analysing the reaction mixture by ^1H NMR these authors found appreciable weakening of the H-1 double doublet at 5.83 ppm with simultaneous appearance of a triplet at 5.35 ppm, which will be due to α,β-anomerisation as described above.

Reaction with other alkyl halides

Reaction of 1 with one equivalent of benzyl bromide yielded 80-85% of 2 within 15 min reaction time. With one equivalent of *p*-methylbenzyl chloride, 80-85% of α-D-glucopyranosyl *p*-methylbenzyl phosphate was formed after 60 min reaction time. The higher reactivity of these reagents is also reflected by formation of side products like dibenzyl phosphate, which occurs more readily than with one equivalent of benzyl chloride.

The scope of this direct alkylation procedure is not restricted to benzyl halides. With 1-bromohexane as the reagent, formation of α-D-glucopyranosyl hexyl phosphate was comparable in rate and selectivity to that of 2, as shown by ^{31}P NMR analysis of the reaction mixture.

The synthesis of surfactants by conversion of 1 with long-chain alkyl halides by this one-step alkylation method is in progress.

ACKNOWLEDGEMENTS

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12. The value of $^{4}J_{H-2,P}$ 2.81 Hz indicated a *trans*-antiplanar arrangement of the five atoms H-2, C-2, C-1, O, and P in aqueous solution[11]. In acetonitrile, however, the bis(tetrabutylammonium) salt of **2** showed no $^{4}J_{H-2,P}$ coupling. As $^{3}J_{H-1,P}$ did not change, indicating that the P-atom is positioned *gauche* to H-1 in both solvents, we conclude that in acetonitrile the phosphate group is oriented *trans* to the ring oxygen in the most abundant rotamer. In the absence of water, there occurs intramolecular hydrogen bonding between the phosphate group and the 2-hydroxyl.
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CHAPTER 5

PREPARATION OF LONG-CHAIN ALKYL D-GLUCOSIDES BY ALCOHOLYSIS OF 1,2:5,6-DI-*O*-ISOPROPYLIDENE-α-D-GLUCOFURANOSE*

5.1 INTRODUCTION

The use of long-chain alkyl D-glucosides (2) as non-ionic detergents having high biodegradability has been claimed[1,2]. Other possible applications include food emulsification[2], solubilisation of phospholipid membranes[3,4], and formation of bilayered vesicles for encapsulation of water-soluble drugs[5]. The liquid crystalline properties of alkyl D-glucosides have been recognised as well[6].

Pure alkyl D-glucosides have been synthesized[7-10] by several procedures employing protection and deprotection steps. Direct acid-catalysed acetalisation of D-glucose with an alcohol, according to the Fischer-method, would seem more economical, however. Using a large excess of alcohol, the α-pyranoside anomer can be isolated from the reaction mixture[11].

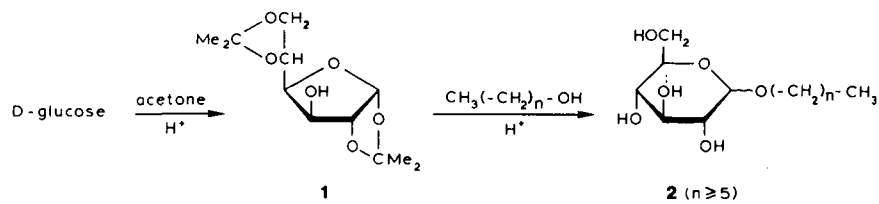
Generally, a large excess of alcohol is not attractive, since separation from the products may be troublesome[1,2,11,12]. Without such an excess, however, alkyl oligo-D-glucosides and oligo-D-glucosides - so far uncharacterised - are produced[2,12] by formation of glucosidic bonds between glucose units. In addition, the low solubility of D-glucose in higher aliphatic alcohols will retard the reaction.

For solubility reasons, D-glucose is occasionally converted into butyl D-glucoside prior to conversion to long-chain alkyl D-glucosides[1,12]. Alternatively, the conversion of D-glucose with acetone might be used as the first step. This acid-catalysed reaction yields 1,2:5,6-*O*-isopropylidene-α-D-glucofuranose (1) as the main product[13,14]. Subsequent acid-catalysed transacetalisation of 1 with long-chain aliphatic alcohols is expected to yield alkyl D-glucosides without solubility problems (Scheme 1).

* A.J.J. Straathof, J. Romein, F. van Rantwijk, A.P.G. Kieboom, and H. van Bekkum, Starch, 39 (1987) 362.

Lower amounts of alkyl oligo-D-glucosides will be formed than by the Fischer-method because the hydroxyl groups are partly protected by isopropylidene groups. Furthermore, an excess of alcohol will eventually result in complete alcoholysis of the isopropylidene groups, so that a separate deprotection step is unneccessary.

This paper deals with the acid-catalysed conversion of 1 with linear aliphatic alcohols. This reaction was studied with 1-butanol as a model alcohol in order to elucidate the pathway of the reaction and to identify the best acid catalyst. The reaction was further optimised using 1-octanol, including a procedure for the isolation of crystalline octyl α-D-glucopyranoside.



Scheme 1

5.2 RESULTS AND DISCUSSION

Mechanistic course

Fig. 1A shows the course of the butanolysis of 1 using macroporous polystyrene ion-exchange resin ($-SO_3H$) as the acid catalyst. An anomeric mixture of butyl D-glucopyranosides (8) is the major product after 6 h at 80 °C, but small amounts of the intermediates 3-7 (*cf.* Scheme 2) are still present. The large number of intermediates reflects the complexity of the reaction.

A detailed picture of the formation of 3-8 at the start of the reaction is given in Fig. 1B. Clearly, 1 is initially converted into 1,2-*O*-isopropylidene-α-D-glucofuranose (3). This compound reaches a maximum concentration after 5 min. Formation of butyl D-glucofuranoside (6) occurs during the first minutes, but its 5,6-*O*-isopropylidene derivative 5 shows up somewhat later. Therefore, the sequence 1 → 5 → 6 cannot be the major pathway for the formation of 6.

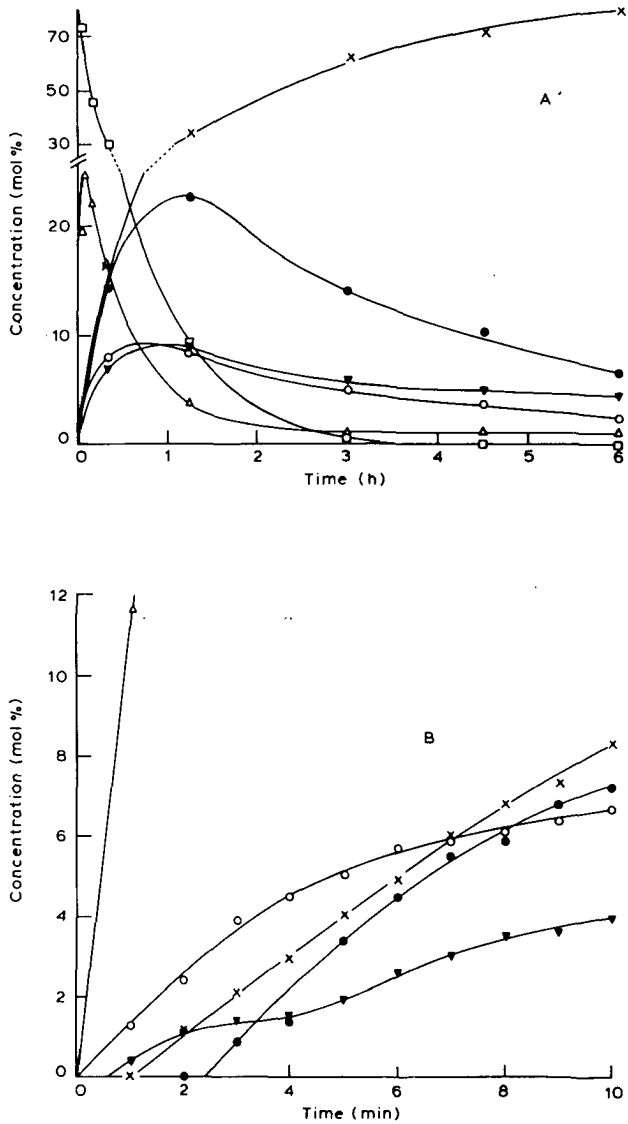


Fig. 1. Course of the reaction of 1 (520 mg, 2 mmol) with 1-butanol (5 mL, 55 mmol) at 80°C in the presence of Dowex MSC-1 ion-exchange resin (-SO_3H, 200 mg). □, 1; △, 3; ○, (4+7); ●, 5; ▼, 6; ×, 8. All concentrations are relative to the initial concentration of 1. A. Overall picture of the reaction. B. Initial formation of 3-8.

It has been shown[15], that D-glucose is formed prior to methyl D-glucofuranoside in the acid-catalysed methanolysis of 3. Moreover, it is well known[16] that methanolysis of D-glucose catalysed by hydrogen chloride proceeds in four steps: fast formation and anomerisation of methyl D-glucofuranoside, followed by slow formation and anomerisation of methyl D-glucopyranoside. The methyl D-glucoside equilibrium consists of α-furanoside, β-furanoside, α-pyranoside, and β-pyranoside in a ratio of 0.6 : 0.9 : 65.8 : 32.7 at either 35 or 64°C[16].

The formation of D-glucose in our reaction mixtures was clearly established. On this basis we propose formation of 8 according to the pathway depicted in Scheme 2: a two-step butanolysis of 1 to D-glucose, followed by formation of furanoside, and isomerisation to pyranoside forms.

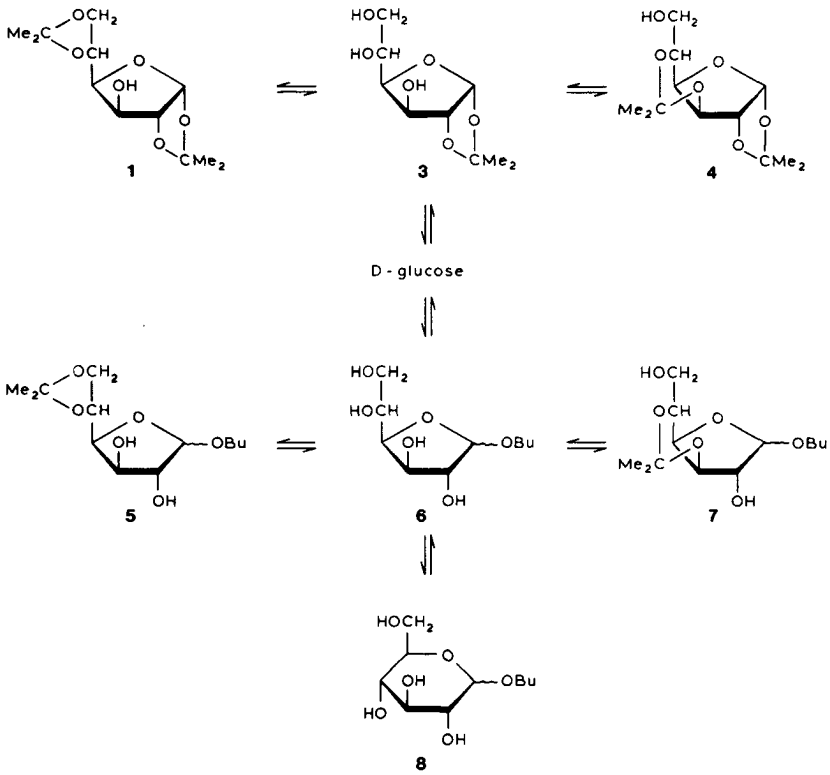
The molar balance of the reaction (Scheme 3) shows that 3 mol of butanol is required for the conversion of 1 mol of 1 into 8. All the water formed will react with the dibutyl acetal of acetone. The acetone thus liberated is assumed to escape largely from the solution by evaporation. In this way the relatively small amounts of water present in the liquid catalysts or adsorbed on the solid catalysts are neglected.

Formation of 5 will proceed by isopropylidation of the 5,6-*O*-position of 6. This is demonstrated by the partial conversion of 3 into 1 under the same conditions. Similarly, 4 and both 5 and 7 will be formed from 3 and 6, respectively.

No unambiguous proof is given for the structure of 7, neither were 4 and 7 separated by HPLC. The appearance of (4 + 7) before 5, 6, and 8 in Fig. 1B, however, is in accordance with the reaction sequence of Scheme 2. Some additional support comes from the constant value of 2.8 found for the ratio of (1 + 5) and (4 + 7) between 3 and 6 h reaction. This indicates that 5,6- and 3,5-*O*-isopropylidene compounds are in a dynamic equilibrium, which may be via 3,5,6-unsubstituted compounds as depicted in Scheme 2, or by direct isomerisation. Isomerisation of 6 into 8, via either endocyclic or exocyclic C-O bond scission[17-19], gradually decreases the proportion of isopropylidene derivatives, since isopropylidation of 8 does not occur.

Variation of acid catalysts

The conversion of 1 into butyl D-glucoside has been studied using various acids as the catalyst, namely sulfuric, methanesulfonic, and tetrafluoroboric acid, a macroporous ion-exchange resin of the sulfonic acid type, and a silica-alumina cracking catalyst (Table 1). The mass balances



Scheme 2

1	+	4 BuOH	⟶	D-glucose	+	2 $Me_2C(OBu)_2$		
D-glucose	+	BuOH	⟶	6	+	H_2O		
$Me_2C(OBu)_2$	+	H_2O	⟶	acetone	+	2 BuOH		
1	+	3 BuOH	⟶	8	+	acetone	+	$Me_2C(OBu)_2$

Scheme 3

Table 1. Effect of the catalyst in the conversion of 1 (520 mg, 2 mmol) with 1-butanol (5 mL, 55 mmol) at 117°C.

Catalyst	Quantity	Time (min)	Concentration (mol%) 1	3	4+7	5[a]	6	8
96% H_2SO_4	4 µL	25	0.0	1.0	4.8	12.2	0.0	55.6
		75	0.0	1.8	3.3	9.3	0.0	61.6
		180	0.0	2.4	2.5	6.5	0.0	76.0
98% $MeSO_3H$	4 µL	25	4.2	1.0	9.3	27.0	5.7	33.6
		75	0.0	0.5	4.8	19.2	3.3	50.1
		180	0.0	1.1	3.7	13.3	3.0	54.0
54% HBF_4[b]	10 µL	25	26.2	8.2	10.5	27.6	8.0	10.6
		75	7.1	1.7	10.4	31.7	8.6	25.1
		180	2.0	0.6	7.7	26.6	9.3	37.8
$-SO_3H$-resin[c]	100 mg	25	3.8	2.9	4.8	14.7	9.3	50.6
		75	0.0	2.5	0.7	4.5	0.0	85.7
		180	0.0	3.5	0.0	0.0	0.0	75.8
Silica-alumina[d]	100 mg	25	42.7	16.1	6.2	6.0	5.3	3.1
		75	35.5	14.5	5.9	9.9	9.5	5.5
		180	17.9	11.9	4.0	10.5	16.1	8.0
Silica-alumina[d]	520 mg	25	3.4	3.1	4.5	16.8	24.6	18.3
		75	0.7	1.5	3.1	11.4	24.1	23.2
		180	0.0	0.7	1.5	5.1	26.1	33.0

[a] Ratio 5α to 5β 2:3. [b] In diethyl ether. [c] Dowex MSC-1. [d] Ketjen cracking catalyst HA-HPV.

were less than 100% because of the formation of D-glucose and some unidentified, probably oligomeric, products. In addition, the response factor of 8 has been used, as an approximation, for 4-7 in the HPLC analyses.

The formation of relatively large amounts of 6 using silica-alumina probably reflects a slow isomerisation of 6 to 8 relative to the formation of 6. The striking amount of 5 which is formed upon using tetrafluoroboric acid is less easy to explain. Additional experiments are required to decide whether 5 is formed from 1 according to Scheme 2 or in a more direct way.

Sulfuric acid seems to be the most active homogeneous catalyst tested, but some decomposition of 8 occurred at higher concentrations of this catalyst. At lower reaction temperature (80°C) no such decomposition was observed, but the yield did not increase (74-77% after 6 h using 5-15 µL acid).

The ion-exchange resin, which seems the best heterogeneous catalyst tested, also promotes decomposition of the products at 117°C (see Table 1). The resin itself is not stable at these conditions either. At 80°C, decomposition problems were absent when < 300 mg of resin was used in our standard formulation. Therefore, the optimum amount of resin was 200 mg for a 6 h reaction (see Fig. 2). The amount of butyl D-glucoside formed at these conditions, which was originally 83.5%, was 80.7% when the reaction was repeated after regeneration of the catalyst, and 78.9% after a second regeneration. A concomitant increase in the amount of intermediates was observed. The resin, which can easily be isolated from the reaction mixture by filtration, thus may be re-used with only a slight loss in catalytic activity. Therefore, this catalyst has been used in the further study of the reaction.

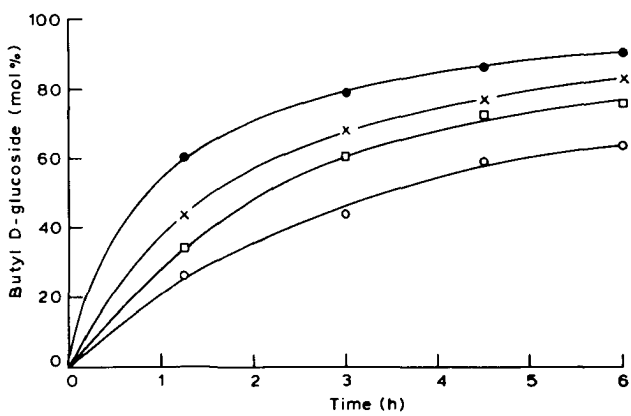


Fig. 2. Formation of butyl D-glucoside by reaction of 1 (520 mg, 2 mmol) in butanol (5 mL, 55 mmol) at 80°C in the presence of Dowex MSC-1 ion-exchange resin (-SO_3H): o, *100 mg;* □, *150 mg;* x, *200 mg;* ●, *300 mg.*

Reaction with octanol

Replacement of 1-butanol by an equal volume of 1-octanol reduces the molar ratio of alcohol to 1 from 55:2 to 32:2. Fig. 3 shows that the yield

of octyl D-glucoside depends on the excess of octanol applied; The highest yield (76%) was obtained at the highest ratio of octanol to 1 (32:1).

During these experiments it was found that octyl α-D-glucopyranoside selectively crystallises from the filtered reaction mixture upon addition of petroleum ether. X-ray analysis of both the α- and β-D-glucopyranoside anomers is in progress[20] to gain some understanding about this phenomenon. A maximum yield of 30% of 98% pure crystalline material was obtained starting with a 32:2 ratio of octanol to 1 (see Fig. 3). The direct crystallisation is an almost ideal isolation method, since it avoids laborious procedures[11] for separating the product from the excess of alcohol.

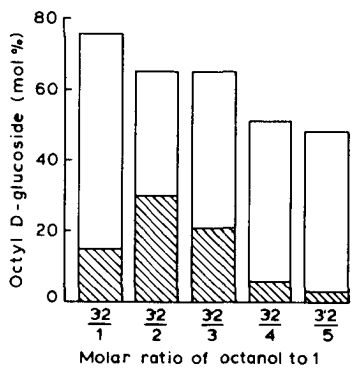


Fig. 3. Amount of octyl D-glucoside formed according to HPLC after 16 h reaction of 1 (1.5 mmol) with 1-octanol (5 mL, 32 mmol) in the presence of Dowex MSC-1 ion-exchange resin (-SO_3H, 150 mg) at 80°C. The shaded parts represent the yield of octyl α-D-glucopyranoside obtained by crystallisation when the reaction was performed on a ten times larger scale.

Recirculation procedure

The yield of crystalline octyl α-D-glucopyranoside from 1 can be improved substantially by recycling the filtrate of the crystallisation. After removal of petroleum ether, this filtrate contains the excess of octanol, uncrystallised octyl α-D-glucopyranoside, and other products of the reaction, mainly octyl β-D-glucopyranoside. After addition of equivalent amounts of 1 and octanol to account for the crystallised glucoside from the former reaction, and starting the reaction for the second time by addition of ion-exchange resin, an anomeric mixture of octyl D-glucosides is obtained

again. Subsequent crystallisation now yields a quantity of octyl α-D-glucopyranoside which is almost equal to that of the first crystallisation. Based on the amounts of **1** and octanol, added for the recirculation procedure, the yield is therefore almost quantitative (98%). The ion-exchange resin can be re-used for this recirculation.

Other alkyl α-D-glucopyranosides

Crystallisation of alkyl α-D-glucopyranosides did not succeed for the reactions of **1** with 1-butanol, 1-hexanol, and 1-hexadecanol under comparable conditions. Using 1-decanol and 1-dodecanol, however, pure α-D-glucopyranosides were obtained in yields of 7 and 11%, respectively, without any optimisation or use of the recirculation procedure mentioned above.

5.3 EXPERIMENTAL

General

1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**1**) and 1,2-*O*-isopropylidene-α-D-glucofuranose (**3**) were prepared by standard methods[13]. The macroporous ion-exchange resin Dowex MSC-1 (20% divinylbenzene, 30-35% porosity, $-SO_3H$) was dried to constant weight at 100°C and 0.1 torr before use. Its capacity was 4.91 x 10^{-3} equiv. H^+/g dry resin according to titration. The silica-alumina cracking catalyst (Ketjen High Alumina - High Pore Volume, Akzo Chemie, The Netherlands, surface area 524 m^2/g) was activated at 700°C before use.

^1H- and ^{13}C NMR spectra were recorded on a Nicolet NT-200 WB instrument at 200 and 50 MHz, respectively. The interpretation of the ^1H NMR spectra was facilitated by double resonance experiments, and a COSY experiment was performed in order to elucidate the ^1H NMR spectrum of 5β.

HPLC analyses were performed at 25°C using a Waters Assoc. M6000A pump, a Waters Assoc. Radial Pak cartridge (8 x 100 mm) custom packed with 10 μm Nucleosil-C18 (Machery-Nagel, Düren, F.R.G.) contained in a Waters Assoc. RCM 100 module, and a Waters Assoc. R401 differential refractometer. The flow of the mobile phase was 1 mL/min. Preparative scale HPLC was performed using a Waters Assoc. Prep LC500 instrument equipped with two 50 x 300 mm reverse-phase cartridges (Waters Ass. PrepPak 500-C18). The flow of the mobile phase was 25-100 mL/min. The HPLC system used for ion-moderated partitioning chromatography has been described in detail elsewhere[21].

Reaction and analysis procedure

A weighed amount of 1 was dissolved in the specified alcohol, which had previously been dried on zeolite NaA pellets. The solution was stirred with exclusion of moisture and the reaction was started by adding the catalyst at the reaction temperature. Heterogeneous reactions were stopped by filtering off the catalyst and washing it with cold methanol. Homogeneous reactions were stopped by pouring the reaction mixture into a cold aqueous solution of equivalent sodium hydrogencarbonate.

The solutions from the butyl D-glucoside reactions were concentrated *in vacuo* below 50°C. After azeotropic distillation with water at the same conditions, 1-butanol was completely removed according to HPLC. A known amount of 1-butanol was added as the internal standard, and the samples were diluted with methanol-water (50:50) for HPLC analysis. Retention times (min): solvent front 3.2, **3** 4.1, **8** 4.9, **6** 5.5, 1-butanol 7.0, **1** 9.1, **4** 11.1, **7** 11.1, 5β 14.5, 5α 16.1. The molar response factor of **8** relative to butanol was also used to calculate the concentration of **4-7**. The concentrations were expressed in mol% relative to the starting concentration of **1**.

The solutions from the octyl D-glucoside reactions were concentrated *in vacuo* and the bulk of the octanol was removed from the samples by azeotropic distillation with water at < 60°C. The samples were dissolved in methanol-water (65:35) for HPLC. Retention times (min): solvent front 3.2, 1 5.8, octyl α- and β-D-glucopyranoside 12.7, 1-octanol 24.4. Octyl D-glucopyranoside was quantified by the external standard method.

Isolation and identification of the intermediates and end-products of the butyl D-glucoside reaction

The glucosidation reaction was performed using 1 (5.2 g), 1-butanol (50 mL), and 96% sulfuric acid (50 µL). After 75 min at 80°C the reaction was stopped and the reaction mixture was analysed by HPLC. The peaks of 1 and 3 were assigned by comparison with authentic samples. The other components present were isolated by preparative HPLC. Several runs were performed using eluents having different ratios of methanol and water: 40:60 for (4+7), 5α, and 5β, 30:70 for 6, while 20:80 afforded separation of 8 into its anomers. Fractions that were > 95% pure according to HPLC were combined and lyophilised after removal of the methanol *in vacuo*. The white solids thus obtained were analysed by ^{1}H- and ^{13}C NMR (see Tables 2 and 3).

Upon comparison with NMR spectra of the corresponding methyl D-glucosides[22], 8α and 8β were identified as butyl α- and β-D-glucopyranoside, respectively, while the isolated fraction of 6 clearly was

Table 2. Assignments of ^1H NMR chemical shifts (A, δ) and ^1H-^1H coupling constants (B, Hz) for compounds 1, 3, 5, 6, and 8[a].

Parameter for	1	3	5α	5β	6α	6β	8α	8β
A, Proton								
Glc H-1	6.03	6.02	5.18	5.03	5.20	5.00	4.92	4.42
Glc H-2	4.70	4.71	4.10	4.13	b	b	3.55	3.22
Glc H-3	4.31	4.33	4.30	4.23	b	b	3.71	3.45
Glc H-4	4.23	4.10	4.32	4.44	b	b	3.41	3.37
Glc H-5	4.43	3.92	4.42	4.48	b	b	3.67	3.45
Glc H-6	4.20	3.81	4.17	4.20	b	b	3.87	3.88
Glc H-6'	4.04	3.64	3.98	4.07	b	b	3.77	3.68
Bu H-α			3.79	3.81	b	b	3.71	3.92
Bu H-α'			3.61	3.61	b	b	3.53	3.66
Bu H-β			1.59	1.59	1.60	1.60	1.60	1.60
Bu H-γ			1.37	1.36	1.37	1.37	1.38	1.37
Bu H-δ			0.87	0.91	0.92	0.92	0.92	0.91
Ip CH_3	1.53[c], 1.47	1.53[c]	1.47	1.48				
	1.37[c], 1.40	1.37[c]	1.39	1.42				
B, Spacing								
H-1, H-2	3.6	3.7	4.2	0	4.1	0	3.7	7.9
H-2, H-3	0.5	0.5	3.4	1.2	b	b	9.8	8.9
H-3, H-4	2.7	2.6	4.7	4.4	b	b	8.8	8.1
H-4, H-5	6.7	8.9	5.0	5.2	b	b	9.8	8.8
H-5, H-6	6.4	2.8	6.5	6.2	b	b	2.3	1.6
H-5, H-6'	5.4	5.9	5.8	5.9	b	b	5.2	5.5
H-6, H-6'	-8.8	-12.0	-8.6	-8.6	b	b	-12.0	-12.1
H-α, H-α'			-9.9	-9.9	b	b	-9.9	-9.9
H-α, H-β			6.6	6.8	b	b	0	6.7
H-α', H-β			6.6	6.6	b	b	6.1	6.7

[a] D_2O was the solvent and TNP the internal standard. [b] Signals not resolved. [c] 1,2-*O*-Isopropylidene group.

a 1:2 mixture of butyl α- and β-D-glucofuranoside. The structures of 5α and 5β were assigned as butyl 5,6-*O*-isopropylidene-α- and -β-D-glucofuranoside, respectively, on account of the correspondence of the anomeric signals with

Table 3. ^{13}C NMR chemical shifts (δ) for compounds 1 and 3-8[a].

Carbon atom	1	3	4	5α	5β	6α	6β	7α[b]	7β[b]	8α	8β
Glc C-1	107.5	107.4	107.3	104.6	110.9	103.5	109.6	103.1	109.8	100.8	104.9
Glc C-2	87.4	87.1	84.6	81.1[c]	85.3[c]	78.2	81.2	75.7	79.4	74.1	75.9
Glc C-3	76.3	76.3	76.0	77.9[c]	77.4[c]	77.1	76.4	77.7	77.2	76.0	78.6
Glc C-4	83.7	82.5	80.5	79.7[c]	82.6[c]	79.3	82.5	77.4	80.1	72.4	72.4
Glc C-5	75.3	71.1	73.8	76.4[c]	76.7[c]	71.2	71.1	72.6	73.5	74.5	78.6
Glc C-6	68.8	66.2	63.4	68.3	68.6	64.7	65.0	62.9	63.0	63.3	63.5
Bu C-α				71.8[c]	71.7[c]	70.7	70.1	70.1	69.4	70.8	73.2
Bu C-β				33.7	33.5	32.4	32.3	32.3	32.3	33.6	33.7
Bu C-γ				21.4	21.4	20.1	20.1	20.1	20.3	21.6	21.2
Bu C-δ				15.8	15.9	14.6	14.6	14.5	14.5	15.9	15.8
Ip acetal C	115.6[d]	115.4[d]	114.7[d]	112.2	111.9			102.9	102.6		
	112.4		103.4								
Ip CH_3	28.4[d]	28.3[d]	27.4[d]	28.2	28.2			25.8	24.6		
	27.9[d]	27.9[d]	26.9[d]	26.7	26.7			25.6	24.2		
	28.4		24.7								
	26.9		24.6								

[a] D_2O was the solvent and TNP the internal standard. [b] Tentative structure and assignments. [c] Assignments may be interchanged. [d] 1,2-*O*-Isopropylidene.

those of 6α and 6β, respectively, and the signals of the 5,6-*O*-isopropylidene rings with those of 1 (see Tables 2 and 3).

Since 4, 7α, and 7β were not separated from each other, their assignment was based on the spectra of the mixture of these compounds. The ^{1}H NMR spectrum showed their anomeric signals: doublets at 6.14 p.p.m. (J 3.8 Hz) and 5.15 p.p.m. (J 4.2 Hz), and a singlet at 5.09 p.p.m., respectively. The ratio of 4, 7α, and 7β was 1:2:3 according to the area of these signals. Consequently, the ^{13}C NMR signals of the three compounds were distinguished from each other by their peak height. In addition to a D-glucofuranose ring, 4 contained two isopropylidene rings, and was identified as 1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose. The downfield shifts of the NMR signals of 4 (and also 1) relative to literature data[14,23] were attributed to solvent effects.

According to ^{13}C NMR, 7α and 7β contained an α- and β-D-glucofuranose ring, respectively, a butyl group, and an isopropylidene ring (seven-membered or six-membered skew[24]). The tentative assignments for 7α and 7β were butyl 3,5-*O*-isopropylidene-α- and -β-D-glucofuranoside, respectively.

The solvent front peak of the HPLC analysis was subjected to ion-moderated partitioning chromatography[21]. The chromatogram showed one major peak, corresponding to D-glucose.

Preparation of octyl α-D-glucopyranoside

The glucosidation reaction was performed using 1 (5.20 g, 20 mmol), 1-octanol (50 mL, 318 mmol), and ion-exchange resin (1.50 g, $-SO_3H$). After 16 h at 80°C the reaction mixture was cooled, filtered, and the resin was washed with petroleum ether (400 mL, b.p. 40-65°C). Octyl α-D-glucopyranoside (1.74 g, 6.0 mmol, 30% based on 1) slowly crystallised from the combined filtrates at -8°C. The purity was 98% according to HPLC. NMR data (CD_3OD): ^{1}H, δ 4.76 (H-1, $J_{1,2}$ 3.8 Hz). ^{13}C, δ 100.1 (C-1), 75.1 (C-3), 73.6 (C-2), 73.6 (C-5), 71.8 (C-4), 62.7 (C-6), 69.1, 33.0, 30.6, 30.6, 30.4, 27.4, 23.7, 14.5 (octyl). After recrystallisation from water: monohydrate[20], m.p. 117 °C, (117°C[6], from water; 124°C[11], from ethyl acetate).

Recirculation procedure

The mother liquor of the reaction described above was concentrated *in vacuo*, thus removing the petroleum ether without distilling off the octanol. To the remaining octanol solution (52 mL) fresh 1 (1.56 g, 6.0 mmol) and

octanol (0.95 mL, 6.0 mmol) were added. The ion-exchange resin used for the previous reaction was added after regeneration by washing with methanol and water, and drying by the standard procedure. After 8 h at 80°C the reaction was stopped. Crystallisation according to the method described above, yielded octyl α-D-glucopyranoside (1.72 g, 5.9 mmol, 98% based on the 1 and octanol added in this recirculation procedure).

Other alkyl α-D-glucopyranosides

The method for the preparation of octyl α-D-glucopyranoside was applied to the reaction of 1 with several linear alcohols. 1-Butanol (6 h reaction), 1-hexanol (8.5 h), and 1-hexadecanol (32 h, petroleum ether was replaced by diethyl ether) showed no crystallisation of glucoside. Decyl α-D-glucopyranoside (0.44 g, 7%) was obtained after 20 h reaction using 1-decanol and crystallisation in the presence of 100 mL petroleum ether. Dodecyl α-D-glucopyranoside (0.78 g, 11%) was obtained using 1-dodecanol (24 h reaction). Petroleum ether was replaced by diethyl ether in order to avoid crystallisation of 1-dodecanol. Decyl and dodecyl α-D-glucopyranoside were identified by their ^{13}C NMR spectra, which were, with exception of the number of methylene groups, virtually identical to the spectrum of octyl α-D-glucopyranoside. After recrystallisation from water: m.p. 138°C (decyl) and 146-147°C (dodecyl), *cf.* 139°C and 151-152°C[11], respectively.

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CHAPTER 6

EFFICIENT PREPARATION OF OCTYL α-D-GLUCOPYRANOSIDE MONOHYDRATE: A RECIRCULATION PROCEDURE INVOLVING WATER REMOVAL BY PRODUCT CRYSTALLISATION*

6.1 INTRODUCTION

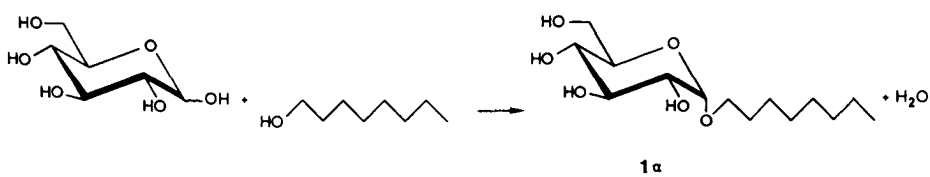
The use of mono- and disaccharides such as D-glucose or sucrose as the hydrophilic moiety of a fatty acid or fatty alcohol based surfactant molecule is attractive for several reasons. The starting materials are renewables that are available in surplusses. They offer a range of non-ionics that show excellent surfactant properties, a good biodegradability, and a low degree of skin and oral toxicity. These carbohydrate-derived surfactants are therefore particularly interesting in applications such as food emulsifiers, cosmetic surfactants and pharmaceutical dispersing agents[1-2]. Their potential use as liquid crystals[3] or biological membrane solubilising detergents[4] has also been noted.

Presently, there are several processes in use for the production of alkyl oligo-D-glucosides, anhydrosorbitol esters, and sucrose esters[5-7]. These processes, however, result in complicated product mixtures and, sometimes, use rather toxic solvents. We have been searching for a one-step procedure which may give a pure carbohydrate-derived surfactant without the use of toxic solvents. In connection with our two-step preparation of alkyl α-D-glucopyranosides from D-glucose via 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose[8], we now have investigated the direct Fischer reaction of a suspension of D-glucose in 1-octanol (Scheme 1). As reported in the patent literature[9-21], several bottle-necks of such a procedure have to be taken into consideration: (*a*) low solubility of D-glucose may be a rate-retarding factor, (*b*) water has to be removed in order to achieve sufficient conversion, (*c*) complicated mixtures are obtained, due to oligoglucoside

* A.J.J. Straathof, H. van Bekkum, and A.P.G. Kieboom, Starch, in press.

formation, (*d*) removal of excess of long-chain alcohol is troublesome, (*e*) coloured by-products are formed, (*f*) unpleasant odour of the product may arise due to traces of alcohol.

The present paper describes a one-step procedure for the preparation of octyl α-D-glucopyranoside (1α). The above-mentioned problems have been overcome by virtue of crystallising 1α monohydrate from the reaction mixture of the Fischer glycosidation, and recycling both the mother liquor and the acidic ion-exchange resin catalyst.



1α

Scheme 1

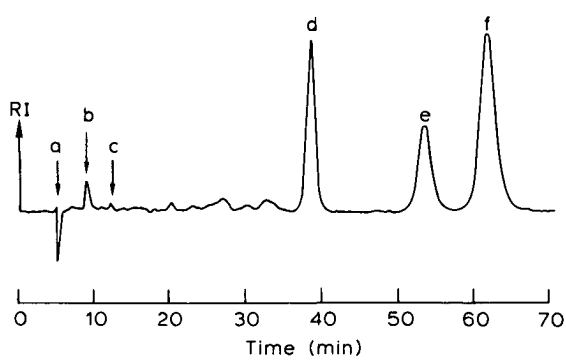


Fig. 1. HPLC of the equilibrium mixture of the Fischer glycosidation (HPX 87H column at 60°C eluted with 0.01 M aqueous trifluoroacetic acid at 0.6 mL/min). a, exclusion peak; b, D-glucose; c, 1,6-anhydro-β-D-glucopyranose; d, methyl isobutyl ketone (internal standard); e, 1β; f, 1α. The other components were not identified.

6.2 RESULTS AND DISCUSSION

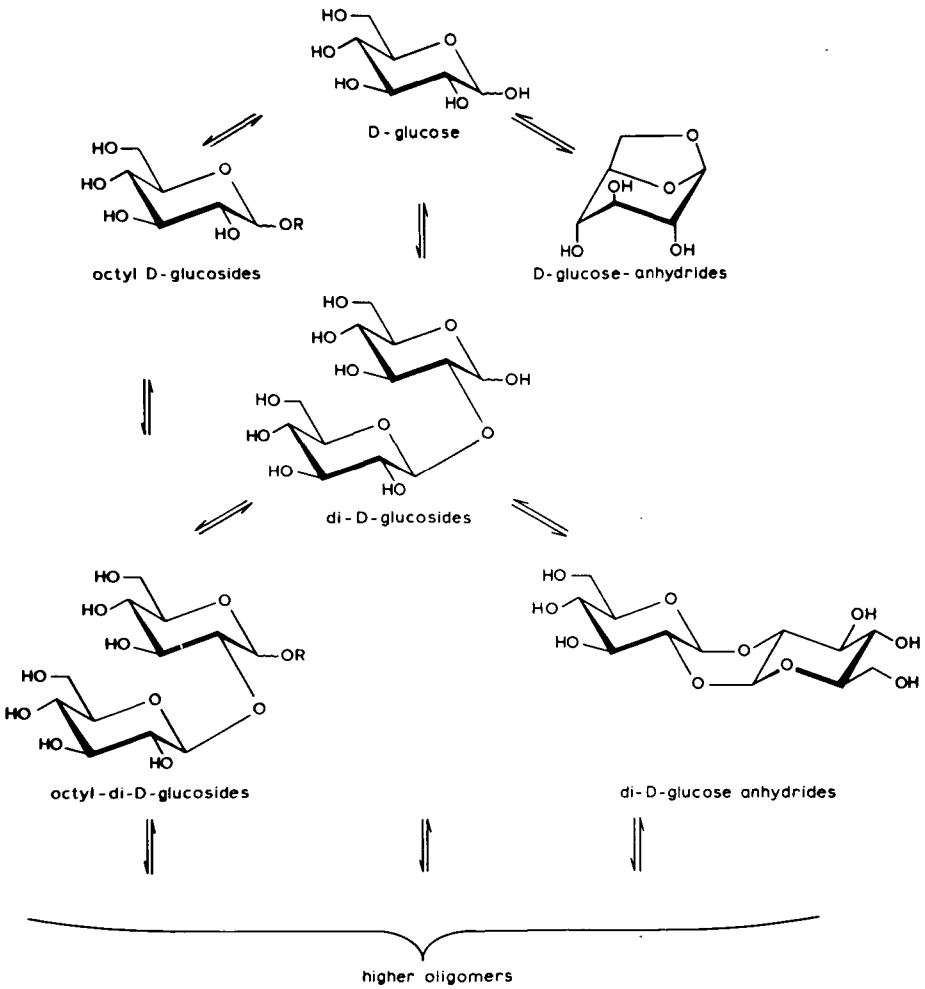
Course of the reaction

Reaction of a suspension of D-glucose (10 mmol) in 1-octanol (159 mmol) in the presence of an ion-exchange resin ($-SO_3H$) at 90°C gave an equilibrium mixture containing ~ 45% octyl α-D-glucopyranoside (1α), ~ 19% octyl β-D-glucopyranoside (1β), ~ 2% D-glucose, ~ 1% 1,6-anhydro-β-D-glucopyranose, and ~ 33% of at least 12 different (oligomeric) compounds (Fig. 1). Apart from a low percentage of octyl D-glucofuranosides the major part of these compounds will consist of reversion products such as oligo-D-glucosides and octyl oligo-D-glucosides[22-23] (Scheme 2). Dioctyl ether was not detected by GCMS, indicating that no etherification reactions besides acetalisation have to be taken into account.

As all reaction products are interconverted under the reaction conditions, selective isolation of the main product 1α in combination with recirculation of the remaining products opens a straightforward method of preparation.

The equilibrium constant for the glucosidation of 1-propanol is about twice that of 2-propanol[24-25]. Assuming a selectivity of glycosidation of primary to secondary hydroxyl groups of 2:1, the presence of ~ 0.5% di-D-glucosides, ~ 16% octyl di-D-glucosides, ~ 4% octyl tri-D-glucosides, and ~ 1% octyl tetra-D-glucosides has to be expected (see Appendix), in accordance with the substantial amount of oligomeric products found experimentally. It may be noted that additional formation of trehaloses[26] and di-D-glucose dianhydrides[27] has not been taken into consideration by the model used.

In contrast to the methanolysis of D-glucose[28], no accumulation of furanoside intermediates was observed during the conversion of D-glucose in 1-octanol. Initially, 1α and 1β were formed in comparable amounts, followed by a relatively slow equilibration to 1α:1β ≈ 2.3:1. Although the solubility of D-glucose in 1-octanol at 90°C is only ~ 1.6 g/L, a suspension of D-glucose (72 g per L 1-octanol) did not show D-glucose particles anymore after ~ 6 h reaction. However, complete equilibration of either such a suspension of D-glucose or a solution of octyl α-D-glucopyranoside monohydrate (124 g/L) under the same conditions required ~ 24 h. Thus, the interconversion of 1α and 1β rather than the low solubility and/or rate of dissolution of D-glucose is a rate limiting factor in obtaining the maximum concentration of 1α.



Scheme 2. Simplified reaction scheme of acid-catalysed reactions of D-glucose in 1-octanol. R = octyl. Furanosides are not shown.

Ion-exchange resin catalysis

Several commercial sulfonated ion-exchange resins of the styrene-divinylbenzene type have been tested for their activity and stability as a catalyst in the glycosidation reaction (Table 1). Although all the resins were reported to be macroporous, BET analysis showed no macropores for Lewatit OC 1052. The low degree of crosslinking of this resin (5% DVB)

Table 1. Characteristics of ion-exchange resins (PS-DVB-SO_3H).

	DVB[a] (%)	Porosity[a] (mL/g)	Swelling[b,c] (mL/mL)	BET (m^2/g)	$\bar{d}_{pore}$ (nm)	$\bar{d}_{particle}$[a] (mm)	Capacity[b] (mequiv. H^+/g)
Lewatit OC 1052	~ 5		2.1	~ 0[b]	< 1[b]	0.3-1.25	5.40
Lewatit SPC 108	~ 8	~ 0.25	1.8	~ 25[a]	~ 40[a]	0.3-1.25	5.21
Lewatit SPC 118	~ 18	~ 0.50	0.7	~ 40[a]	~ 65[a]	0.3-1.25	5.01
Dowex MSC-1	~ 20	0.30-0.35	0.5	~ 35[a,b]	~ 17[b]	0.3-0.9	4.91

[a] According to product information from Bayer (Lewatit) and Dow Chemical (Dowex).

[b] Determined after drying to constant weight. [c] At 90°C, in 1-octanol.

apparently does not prevent the macropores to collapse upon drying, whereas the other resins are rigid enough to maintain their macroporous structure in vacuum. The three-dimensional structure will be dependent on the reaction conditions. In 1-octanol at 90°C appreciable swelling occurred within a few minutes. The extent of swelling was inversely proportional to the degree of crosslinking of the resin. At 20°C, the swelling time exceeded 24 h.

The capacities of the resins correlate well with complete monosulfonation of the phenyl residues without appreciable substitution of the phenylene residues in the matrices.

As a catalyst in the reaction of D-glucose in 1-octanol, the resins show a comparable behaviour (Fig. 2). The isomerisation of α-pyranosides and β-pyranosides is slow and α/β-equilibration is complete only at ~ 100% conversion. Using the D-glucose half-life in the presence of 0.36 equiv. H^+ as measure of the catalyst activity, Lewatit SPC 118 proved to be a more active catalyst (~ 0.6 h) than the other resins (~ 2 h). The relatively high BET surface and $\bar{d}_{pore}$ of Lewatit SPC 118 qualitatively explain its high activity. In test reactions, however, it showed a low mechanical stability.

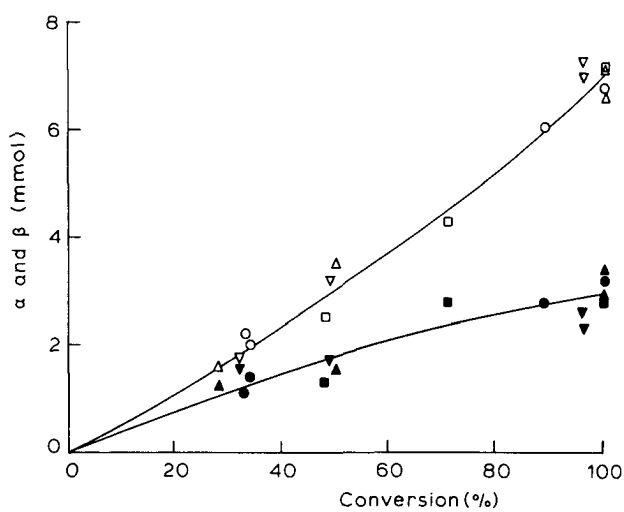


Fig. 2. Formation of mono- and oligo-α-D-glucopyranosides (open symbols) and -β-D-glucopyranosides (closed symbols) in the conversion of D-glucose (10 mmol) in 1-octanol (25 mL) in the presence of different ion-exchange resins (3.6 mequiv. -SO_3H) according to ^1H NMR: ○ *and* ●, *Lewatit OC 1052;* ▽ *and* ▼, *Lewatit SPC 108;* □ *and* ■, *Lewatit SPC 118;* △ *and* ▲, *Dowex MSC-1.*

Lewatit OC 1052 and Dowex MSC-1 were much more stable and Lewatit SPC 108 showed almost no decrease in particle size when subjected to the reaction conditions. Therefore the latter resin was used in further experiments.

The drying procedure used has been reported to reduce the water content of the ion-exchange resins to < 1% w/w[29], so the amount of water introduced in the reaction mixture is negligible with respect to the amount of water formed during the glycosidation reaction.

Crystallisation of 1α

It was found that 1α can be crystallised selectively from the reaction mixture by the addition of 8 volumes of petroleum ether (b.p. 40-65°C). The yield of 1α was 27%, *i.e.* 60% of the 45% 1α present at equilibrium. By applying a recirculation procedure (see below) the yield could be raised to > 95%.

Recently, the crystal structure of anhydrous 1α and the monohydrate of 1α have been elucidated[30]. XRD analysis showed that the present method yields 1α monohydrate, a felicitous circumstance since product isolation simultaneously removes the molar amount of water during its formation. This means that additional water removal from the remaining reaction mixture in a recirculation procedure is not required. In this respect it may be noted that drying of the reaction mixture with zeolite KA resulted in crystalline anhydrous 1α, but in a lower yield.

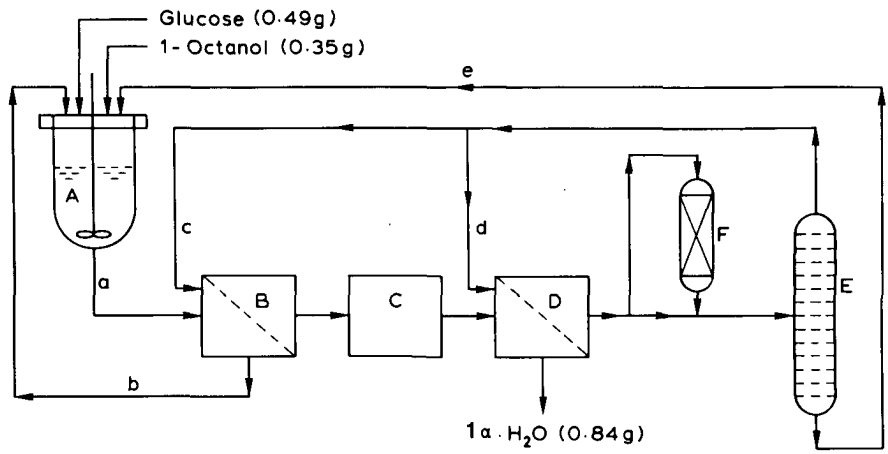
The preferred crystallisation of 1α relative to 1β is assumed to originate from the better crystal packing of 1α[31].

Recirculation procedure

Scheme 3 shows the recirculation procedure used for the preparation of 1α monohydrate. After 6 h reaction at 90°C (A) the ion-exchange resin was filtered off, washed with petroleum ether (B) and recirculated to the reaction vessel (A). Addition of petroleum ether to the filtrate resulted in crystalline 1α monohydrate (C), which was isolated by filtration (D). Petroleum ether was removed from the mother liquor by distillation (E) and the bottom recirculated to the reaction vessel (A). Equivalent amounts of D-glucose and 1-octanol were added to account for the crystalline 1α monohydrate obtained. The resulting mixture was reacted in the presence of the recirculated ion-exchange resin. This cycle was repeated three times without any decrease in product yield. Thus, the first cycle yielded 27% crystalline 1α monohydrate (based on 10 mmol D-glucose) and the second, third, and fourth cycle each gave a quantitative yield of 1α (based on the

additional 2.7 mmol D-glucose and 1-octanol per cycle). After these four cycles no decrease in capacity of the Lewatit SPC 108 resin was found. Although there was some colour formation in the solution during the reaction step of each cycle, 1α monohydrate was obtained as white crystals. HPLC showed no accumulation of other compounds. Moreover, the mother liquor was easily decolourized by elution over a bed of active carbon (F).

Application of the recirculation procedure to the preparation of longer alkyl α-D-glucopyranosides is under present investigation.



Scheme 3. Process scheme (batch) for the preparation of 1α monohydrate. A, reaction; B, filtration; C, crystallisation; D, filtration; E, distillation; F, decolourization; a, equilibrium mixture from the 6 h reaction of 1.8 g D-glucose and 25 mL 1-octanol at 90°C; b, ion-exchange resin (Lewatit SPC 108, 0.69 g); c, petroleum ether (200 mL) for washing and crystallisation; d, petroleum ether (20 mL) for washing; e, mother liquor of crystallisation (~ 22 g, mainly 1-octanol).

6.3 CONCLUSIONS

The recirculation procedure described allows the one-step preparation of pure and crystalline octyl α-D-glucopyranoside (1α) monohydrate in quantitative yield from D-glucose and 1-octanol. No toxic or expensive cosolvents are required and the acidic ion-exchange resin can be repeatedly used as the catalyst. There is no increase of the water content in the reactor during the recirculation as the reaction water is removed through

the crystallisation step. Disadvantages are the high recirculation ratio required (3) and the time-consuming crystallisation process.

6.4 EXPERIMENTAL

Materials

Anhydrous D-glucose was obtained from Merck, 1-octanol from Baker, and Lewatit and Dowex ion-exchange resins from Bayer and Aldrich, respectively. Petroleum ether (b.p. 40-65°C) contained 36% pentane, 6% cyclopentane, 2% 2,2-dimethylbutane, 8% 2,3-dimethylbutane, 36% 2-methylpentane, 10% 3-methylpentane, and 2% hexane according to ^{13}C NMR. Octyl β-D-glucopyranoside (Sigma) was anhydrous according to elemental analysis.

Ion-exchange resins

The ion-exchange resins were washed by elution with 0.5 M aqueous sodium carbonate, water, 1.0 M aqueous hydrochloric acid, and water, respectively, and dried to constant weight at 100°C and 3 mm Hg. Capacities were determined by adding an excess of 0.1 M aqueous sodium hydroxide and back-titrating with 0.1 M aqueous hydrochloric acid. Swelling was determined at 90°C by partially filling a 10 x 400 mm column with resin and adding 1-octanol.

HPLC analysis

HPLC (Fig. 1) was performed on a M6000A system equipped with a R401 refractive index detector (Waters Assoc.). An Aminex HPX 87H column (200 x 9 mm, Bio-Rad) at 60°C was eluted with 0.01 M aqueous trifluoroacetic acid at 0.6 mL/min. The molar response factor of 1β relative to 1α was 0.90. The presence of 1,6-anhydro-β-D-glucopyranoside in the samples was confirmed by additional analysis on an Aminex HPX 87C column at 60°C with water as the eluent.

NMR

^{1}H NMR spectra were recorded on a Nicolet NT-200 WB instrument at 200 MHz. 2-Chloro-1,3,5-trinitrobenzene was used as internal standard. Hydroxyl groups were deuterated by addition of D_2O and evaporation. The spectra were recorded in acetone-d_6, and the signals of *mono*- and oligo-α-D-glucopyranosides (δ = 4.7-4.9 ppm) and -β-D-glucopyranosides (δ = 4.2-4.5 ppm), and of the internal standard (δ = 9.2-9.3 ppm) were integrated. ^{13}C

NMR of petroleum ether (b.p. 40-65°C) was performed at 50 MHz with a pulse delay time of 120 s. The alkanes were identified by comparison with reference spectra after editing with a DEPT experiment[30].

Solubility determination

A suspension of anhydrous D-glucose in 1-octanol was stirred at 90°C for 3 h. After 3 h settling, a 10 mL sample of the 1-octanol solution was extracted with water. From the amount of D-glucose in the combined aqueous layers (HPLC analysis) a solubility of 1.6 g of D-glucose per L 1-octanol was obtained.

Reaction procedure

A suspension of anhydrous D-glucose (1.80 g; 10 mmol) and ion-exchange resin (3.60 mequiv. H^+; ~ 0.7 g) in 1-octanol (25 mL; 159 mmol) was stirred in a closed vessel at 90°C. The samples taken after settling were freed from 1-octanol by azeotropic distillation with water at 50°C, 20 mm Hg. Preparative experiments were carried out with Lewatit SPC 108 as the catalyst (690 mg). After 6 h reaction the mixture was cooled and filtered. The resin was washed with petroleum ether (200 mL, b.p. 40-65°C) and 1α monohydrate (0.84 g. 2.7 mmol) was obtained from the combined filtrates by crystallisation at -8°C during one week. The crystals were washed with petroleum ether (20 mL) and the mother liquor was concentrated *in vacuo* in order to remove petroleum ether. D-Glucose (2.7 mmol), 1-octanol (2.7 mmol), and the recovered ion-exchange resin were added to the concentrated mother liquor. The resulting suspension was stirred for 6 h at 90°C. The same work-up procedure as described above gave again crystalline 1α monohydrate (0.84 g; 2.7 mmol). The crystals were identified by X-ray diffraction. ^{13}C NMR data and clearing point (117°C) were identical to literature data[8].

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APPENDIX

Model for the estimation of the extent of D-glucose oligomerisation

In this model, any oligoglucoside present at equilibrium is indicated by G^i_nOX. G^i_n is an oligoglucosyl group (linear or branched), containing n D-glucose residues. For X = H, G^i_nOX is an oligoglucoside having a free anomeric hydroxyl group, and for X = R, G^i_nOX is an octyl oligoglucoside (R = octyl). The occurrence of trehaloses and anhydrides has not been taken into account.

The number of glycosidic bonds to aglycon groups that are residues of secondary alcohol groups is indicated by the superscript i. Thus, maltotriose is indicated by G^2_3OH. For $n < 5$, Table 2 shows the number (Y^i_n) of different isomers of G^i_nOX for each real value of i. (The model does not distinguish furanoside, pyranoside, α, and β isomers.)

It is assumed that the glycosidation equilibrium constant will be K for any primary alcohol group as the aglycon and $^1/_2$ K for any secondary alcohol group[24-25]. The formation of G^0_1OR according to

$$G^0_1OH + HOR \underset{}{\overset{K}{\rightleftharpoons}} G^0_1OR + H_2O \qquad (1)$$

gives

$$\frac{G_1^0OH}{H_2O} = \frac{G_1^0OR}{K \cdot HOR} \qquad (2)$$

Substitution of (2) in

$$G_1^0OH + G_1^0OR \overset{K.Y_2^0}{\rightleftharpoons} G_2^0OR + H_2O \qquad (3)$$

with $Y_2^0 = 1$ leads to

$$G_2^0OR = \frac{G_1^0OR}{HOR} \cdot G_1^0OR \qquad (4)$$

and in

$$G_1^0OH + G_1^0OR \overset{1/2\ K.Y_2^1}{\rightleftharpoons} G_2^1OR + H_2O \qquad (5)$$

leads to

$$G_2^1OR = \frac{Y_2^1}{2} \cdot \frac{G_1^0OR}{HOR} \cdot G_1^0OR \qquad (6)$$

Using the general reaction equation (a = 0 or 1)

$$G_1^0OH + G_{n-1}^{i-a}OX \overset{K^*}{\rightleftharpoons} G_n^iOX + H_2O \qquad (7)$$

with

$$K^* = (1/2)^a \cdot K \cdot Y_n^i / Y_{n-1}^{i-a} \qquad (8)$$

the general equation for G_n^iOX becomes

$$G_n^iOX = Y_n^i \cdot (1/2)^i \cdot \left(\frac{G_1^0OR}{HOR}\right)^{n-1} \cdot G_1^0OX \qquad (9)$$

The sum S_nOX of the molar amount of glucose units in species containing n glucose units at equilibrium is given by

$$S_nOX = n \cdot \sum_{i=0}^{n-1} G_n^iOX \qquad (10)$$

while the total amount of glucose units T equals

$$T = \sum_{n=1}^{\infty} (S_nOH + S_nOR) \qquad (11)$$

When concentrations are expressed as mol % of the initial amount of D-glucose, the known concentrations at equilibrium are: GOH ~ 2%, GOR ~ 70% (pyranosides and furanosides), and HOR ~ 1500%. The values found for G_n^iOX and S_nOX with these numbers are shown in Table 2. Summation gives T = 94%.

Table 2. Percentages of various oligo-D-glucosides and octyl oligo-D-glucosides present at equilibrium according to the model described in the Appendix.

n	i	Y_n^i	G_n^iOH (%)	G_n^iOR (%)	S_nOH (%)	S_nOR (%)
1	0	1	2	70	2	70
2	0	1	0.093	3.267	0.47	16.33
2	1	3	0.140	4.900		
3	0	1	0.004	0.152		
3	1	9	0.020	0.686	0.11	3.89
3	2	12	0.013	0.457		
4	0	1	0.000	0.007		
4	1	18	0.002	0.064		
4	2	66	0.003	0.117	0.03	0.95
4	3	55	0.001	0.049		

CHAPTER 7

A NOTE ON THE APPLICATION OF MICROWAVE TECHNOLOGY TO THE PREPARATION OF 1,6-ANHYDROGLUCOSE FROM (1→4)-GLUCANS

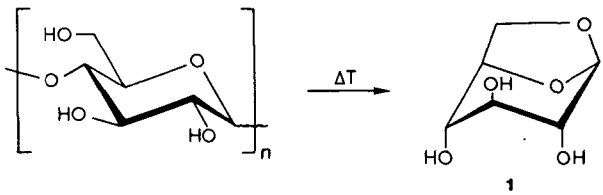
7.1 INTRODUCTION

1,6-Anhydro-β-D-glucopyranose (1) is a useful synthon in carbohydrate chemistry[1-2], *e.g.* in the preparation of long-chain alkyl D-glucosides, which are of interest because of their surface-active and liquid crystalline properties[3]. Amongst the many synthetic routes to 1 published[1], the thermolysis of starch[4] (or cellulose[5]) is the most economical and straightforward method. The reaction is carried out at 300-400°C under a vacuum of ~ 0.1 mm Hg in order to remove 1 from the reaction mixture by sublimation before it will be subject to decomposition[4]. Because of the poor heat transfer by starch, control of the reaction temperature is very difficult and upon conventional heating specially designed apparatus has to be applied for upscaling[6].

As the thermolysis reaction might be better controllable when uniform heating of the starch is applied, microwave heating might be useful. In an elegant study, Allen *et al.*[7] reported on the formation of 1 upon microwave heating of small samples (< 3 g) of dry cellulose. In preparative organic chemistry some examples are known in which the use of a microwave oven can reduce the reaction time as well as the formation of side-products considerably[8-10].

Upon microwave heating the radiation energy is dissipated principally by the orientation of dipoles parallel to the electromagnetic field. Since the relaxation frequency of the orientated dipoles (~ 40 GHz for water, but probably somewhat lower for hydroxyl and other functional groups) has the same order of magnitude as the radiation frequency (2.45 GHz for most commercial microwave ovens), the kinetic energy of the molecules increases and the temperature rises rapidly and uniformly[11].

We have explored the application of microwave heating to the thermolysis of starch and related (1→4)-D-glucans for a rapid and simple preparation of 1.



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Scheme 1.

7.2 RESULTS AND DISCUSSION

Upon irradiation of starch in a household-type microwave oven the thermolysis reaction was found to start within a few minutes in the centre of the sample. Because of the evolution of gases such as water vapour considerable swelling of the starch, which turns into a brown mass, occurred. It appeared that 1 was formed together with glucose, fructose, 5-hydroxymethylfurfural, and oligomeric products.

In a typical experiment, 25 g starch was heated for 7.5 min (2.45 GHz, 400 W) and quenched with 100 mL water. After addition of 300 mL 2-propanol the solids were filtered off and the filtrate was concentrated to dryness. The resulting solid was extracted with 100 mL ethanol and the extract concentrated to a syrup containing 0.40 g (1.7%) 1. Crystallisation from acetone yielded 1 (0.25 g; 1.0%).

Recirculation of the polymers precipitated by 2-propanol during the working-up of the 25 g starch reaction mixture yielded 0.12 g of 1, thus raising the total yield from 1.7 to 2.2%.

If there would be no heat loss to the surroundings, the irradiation time at constant oven power would have to be increased linearly with the size of the sample. However, ~ 5 and ~ 15 min irradiation was required to accomplish complete reaction for 10 and 80 g starch, respectively, whereas no reaction occurred with ⩽ 2 g starch in the equipment used, demonstrating the relatively larger heat loss to the surroundings for smaller samples.

The yield of 1 from different (1→4)-glucans varied from 0.65-1.7% (Table 1). No correlation was found with the chemical structure, degree of polymerisation, or the water content of the (1→4)-glucans used.

On the other hand, Fig. 1 suggests that the density of the (1→4)-glucans is of importance. Thus, potato starch which has a relatively high crystallinity, gave better yields than amylose and amylopectine samples having a much lower density. Because of the relatively small volume and outer surface of the high-density starch sample, its heat loss will be lower and it will reach a higher temperature. The thermolysis reaction to 1 seems to be more favourable at a higher temperature.

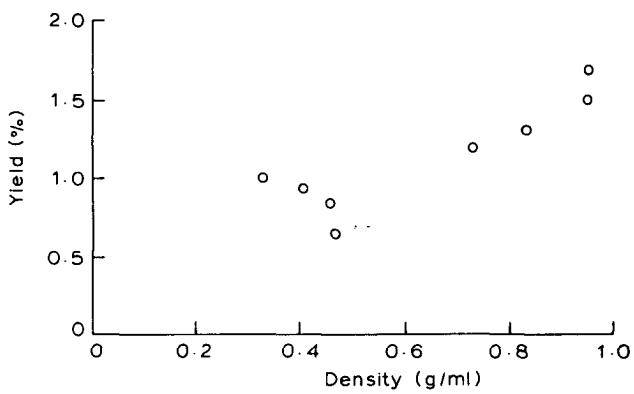

Fig. 1. Yield of 1,6-anhydroglucose upon microwave degradation of 25 g (1→4)-D-glucan as a function of the density of the sample (7.5 min irradiation, 2.45 GHz, 400 W).

When using 25 g starch which was dried before at 100°C and 3 mm Hg the yield of 1 hardly decreased. Thus, the presence of water is not of major importance.

In order to increase the yield substantially, 1 should have to be removed during the reaction, *e.g.* by vacuum sublimation as in the conventional thermolysis of starch. Owing to the formation of a plasma upon microwave irradiation of a high vacuum, the latter method is not applicable. For obtaining a small amount of 1 (~ 1 g) the ease of the present method outweights the low yield, since the starting material is inexpensive. The reaction is under study using temperature-controlled power input (up to

2000 W) in order to raise the yield and to determine the scope and limitation of this method.

Table 1. Yield of 1 upon microwave irradiation of various (1→4)-D-glucans[a].

Substrate	$\bar{n}$ [b]	Water content[c] (% w/w)	Density (g/mL)	Yield of 1[d] (mg)	(% w/w)
cellulose	2,800	6.6	0.41	220	0.94
starch	14,000	4.2	0.95	398	1.7
starch	14,000	0.0	0.95	382	1.5
amylopectin	2,000,000	11.0	0.33	226	1.0
amylose	3,000	13.1	0.73	262	1.2
maltodextrin 2[e]	50	9.5	0.46	189	0.84
maltodextrin 10[e]	10	7.5	0.47	150	0.65
β-cyclodextrin	7	10.5	0.83	280	1.3

[a] 25 g, 7.5 min irradiation time. [b] Chain average degree of polymerisation (ref. 12). [c] Water content at the start of the reaction (defined by weight loss at 100°C, 3 mm Hg). [d] According to HPLC after work-up. [e] Paselli maltodextrins.

7.3 EXPERIMENTAL

Materials

Amylopectin, amylose, β-cyclodextrin and the maltodextrins Paselli MD 2 and MD 10 were gifts from Avebe (Veendam, The Netherlands). Native potato starch and microcrystalline cellulose were obtained from Merck. The water contents were defined by the weight loss upon heating at 100°C and 3 mm Hg (constant weight was reached within 24 h). The densities were determined in a graduated cylinder after settling of the solids upon vibration.

Procedure

The substrate (25 g) in a 200 mL beaker was heated in a Sharp microwave oven (model R4060(W), 2.45 GHz, 400 W). After 7.5 min the beaker was removed and the reaction was quenched by the addition of water (100 mL). The liquid

was stirred for 5 min and 2-propanol (300 mL) was added from a dropping funnel during 20 min. The undissolved polymer and the polymer thus precipitated were filtered off and the filtrate was concentrated *in vacuo*. The resulting solid was heated with ethanol (100 mL) under reflux for 30 min. The ethanol extract was concentrated to a syrup of crude **1** that was analysed by HPLC (Aminex HPX 87C column at 60°C, eluted with 0.6 mL/min water[13]). Crystallisation from acetone gave **1**, m.p. 173-177°C (*cf.* 178°C[4]). ^{1}H NMR data were identical to literature data[14].

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CHAPTER 8

KINETICS AND MECHANISM OF THE ACID-CATALYSED BUTANOLYSIS OF 1,6-ANHYDRO-β-D-GLUCOPYRANOSE

8.1 INTRODUCTION

Long-chain alkyl α-D-glucopyranosides are of interest because of their surface-active[1] and liquid-crystalline[2] properties. In this respect we have recently described the acid-catalysed alcoholysis of 1,2:5,6-di-*O*-isopropylidiene-α-D-glucopyranose[3] and the acetalisation of D-glucose[4] with $\geq C_8$ fatty alcohols. Upon liberation of acetone and water, respectively, a mixture of alkyl D-glucosides was formed from which the α-D-pyranoside isomer could be isolated by direct crystallisation. As the rate of formation of this thermodynamically more favourable isomer appeared to be relatively low, complete equilibration of alkyl D-pyranoside isomers in order to obtain a maximum yield of the α-D-pyranoside isomer required additional reaction time. Therefore we decided to study the possibly selective kinetic formation of alkyl α-D-glucopyranosides by acid-catalysed alcoholysis of 1,6-anhydro-β-D-glucopyranose (1), available from starch[5,6] or cellulose[6,7] thermolysis.

In the past, the alcoholysis of 1 has hardly been studied. Although in an early publication the hydrogen chloride-catalysed methanolysis reaction of 1 was supposed to yield initially methyl β-D-glucopyranoside[8], later reports on the acid-catalysed alcoholysis of 1 and its derivatives gave no further mechanistic information[9-13]. On the other hand, the Lewis acid-catalysed polymerisation of derivatives of 1 has been intensively studied[14,15]. Highly stereoregular (1→6)-α-D-glucopyranan was obtained by the phosphorous pentafluoride-catalysed reaction of the 2,3,4-tri-*O*-methyl derivative of 1 in dichloromethane at -78°C. The concurrent formation of β-linkages under less stringent conditions has been attributed to premature chair inversion of a cationic $^1C_4$ transition state complex[14,15]. In this paper we present a kinetic and mechanistic study of the sulfuric acid-catalysed conversion of 1 in 1-butanol as a model alcoholysis reaction. For

comparison, the behaviour of butyl β-D-glucopyranoside (2β) under the same reaction conditions has been studied as well.

8.2 RESULTS

Figs. 1 and 2 show the course of the homogeneous reaction of 1,6-

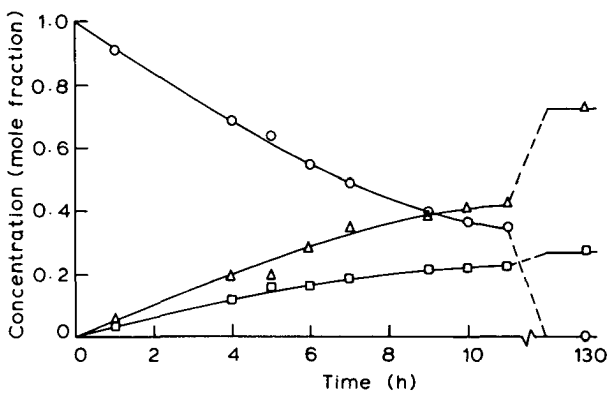


Fig. 1. Acid-catalysed conversion of 1 (○) *into an equilibrium mixture of 2α* (△) *and 2β* (□) *(50 mM 1, 5 mM sulfuric acid, 1-butanol, 80°C).*

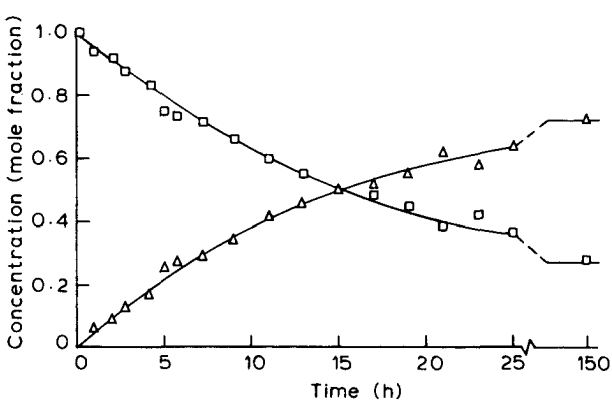
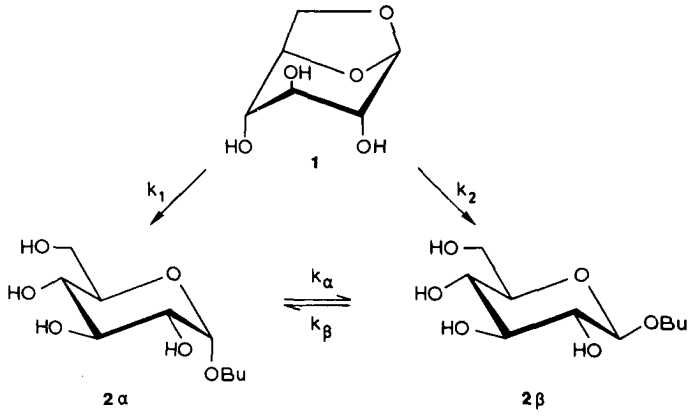


Fig. 2. Acid-catalysed conversion of 2β (□) *into an equilibrium mixture with 2α* (△) *(50 mM 2β, 5 mM sulfuric acid, 1-butanol, 80°C).*



Scheme 1

anhydro-β-D-glucopyranose (1) and butyl β-D-glucopyranoside (2β), respectively, catalysed by sulfuric acid in 1-butanol at 80°C. A clean mixture of 2α and 2β was obtained, accounting for the total molar balance according to HPLC. (In the presence of traces of water, however, formation of a few % butyl D-glucofuranoside occurred.) The reaction from either 1 or 2β finally yielded an equilibrium mixture of 73% 2α and 27% 2β.

The pseudo first-order reaction rate constants k_1, k_2, k_α, and k_β (Scheme 1) have been determined in the following way. In the isomerisation of 2β its rate of conversion is given by

$$-\frac{d[2\beta]}{dt} = k_\beta.[2\beta] - k_\alpha.[2\alpha] \qquad (1)$$

or, in the integrated form, by $y = (k_\alpha + k_\beta).t$, with

$$y = -\ln ((k_\alpha/k_\beta + 1).[2\beta] - k_\alpha/k_\beta) \qquad (2)$$

The kinetic data (Fig. 3) yield $(k_\alpha + k_\beta) = (21.5 \pm 1.0) \times 10^{-6}\ s^{-1}$, from which k_α and k_β may be obtained using $[2\beta]/[2\alpha] = k_\alpha/k_\beta = 0.37$ (Table 1).

The rate of conversion of 1 is given by

$$-\frac{d[1]}{dt} = (k_1 + k_2).[1] \qquad (3)$$

or, in the integrated form, by

$$\ln[1] = -(k_1 + k_2).t \qquad (4)$$

From the experimental data (Fig. 4) an overall rate constant $(k_1 + k_2) = (27.5 \pm 1.0) \times 10^{-6}\ s^{-1}$ is obtained.

The rate of formation of 2β during the butanolysis of 1 is given by

$$\frac{d[2\beta]}{dt} = k_2.[1] + k_\alpha.[2\alpha] - k_\beta.[2\beta] \qquad (5)$$

which can be integrated to $z = (k_2 - k_\alpha).x$, with

$$z = [2\beta] - \frac{k_\alpha}{k_\alpha + k_\beta}\,(1 - \exp(-(k_\alpha + k_\beta).t)) \qquad (6)$$

$$x = \frac{\exp(-(k_1 + k_2).t) - \exp(-(k_\alpha + k_\beta).t)}{k_\alpha + k_\beta - (k_1 + k_2)} \qquad (7)$$

The experimental data (Fig. 5) give $(k_2 - k_\alpha) = (5.1 \pm 0.3) \times 10^{-6}\ s^{-1}$, from which k_1 and k_2 are obtained using $(k_1 + k_2)$ and k_α (Table 1).

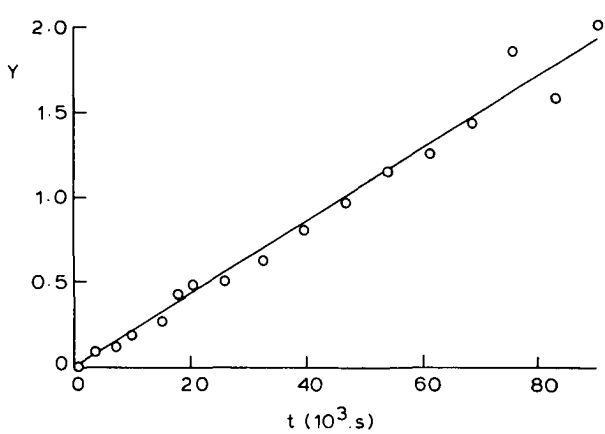


Fig. 3. Kinetic relation $y = (k_\alpha + k_\beta).t$ for the conversion of 2β by butanolysis (see Equation 2).

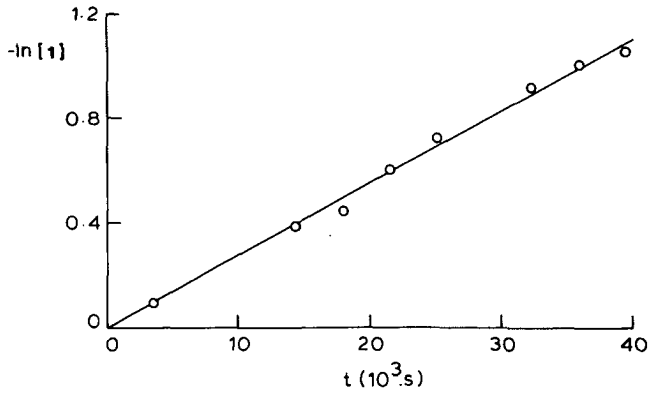


Fig. 4. Kinetic relation ln[1] = -(k_1 + k_2).t for the conversion of 1 by butanolysis.

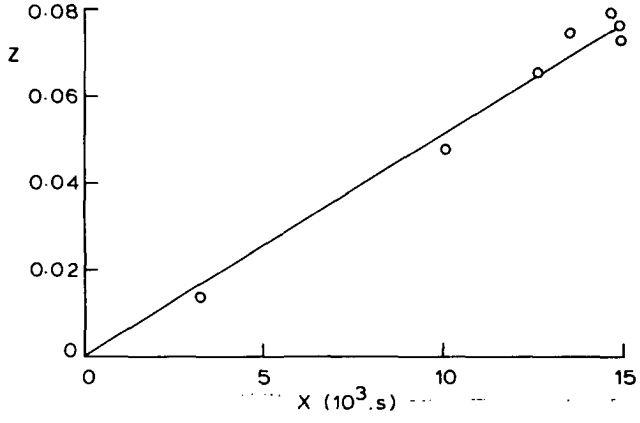


Fig. 5. Kinetic relation z = (k_2 - k_α).x for the formation of 2β during the butanolysis of 1 (see Equations 6-7).

8.3 DISCUSSION

The mechanism of the acid-catalysed anomerisation of butyl D-glucopyranosides in 1-butanol will be comparable to that of the methyl homologues in methanol. It has been shown that in tetradeuteromethanol methyl β-D-glucopyranoside is not converted into its α-anomer but into trideuteromethyl α-D-glucopyranoside (> 80%) and its β-anomer (< 20%) at the initial

Table 1. Pseudo first-order reaction rate constants for the butanolysis of 1, 2α and 2β (Scheme 1)[a].

Rate constant	10^{-6} s^{-1}
k_1	16.5 ± 1.1
k_2	11.0 ± 0.4
k_α	5.8 ± 0.5
k_β	15.7 ± 0.6

[a] Reaction conditions: 50 mM substrate and 5 mM H_2SO_4 in 1-butanol at 80.0°C.

stage of the reaction[16]. Therefore, following protonation of the exocyclic oxygen atom and loss of methanol the formation of an intermediate D-glucopyranosyl oxycarbonium ion (3) has been proposed[16]. A study of the solvolysis of D-glucopyranosyl derivatives in mixtures of ethanol and 2,2,2-trifluoroethanol revealed that the transition state for α- or β-D-glucoside formation contained the leaving group[17]. The preferred conformation of this transition state will be probably a $^4H_3$-like half-chair (*cf.* Scheme 2), solvated at the α- and β-face of C-1 by an alcohol.

Such a transition state together with a low rate of exchange with bulk solvent molecules results in a clean S_N2 reaction. In the case of a high rate of exchange an S_N1 reaction will be observed. Previous studies[16-18] indicate that the rates of exchange and reaction are of the same order of magnitude.

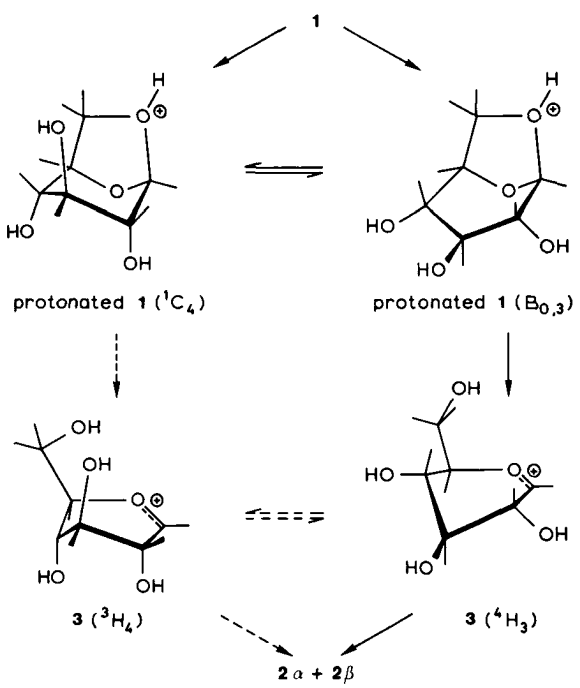
In the butanolysis of 1, the leaving group cannot diffuse into bulk solvent. Apparently the bond between O-6 and C-1 is selectively broken, since pyranosides rather than septanosides are formed. Upon protonation of O-6 and bond cleavage the $^1C_4$ conformation of 1 will be converted into an oxycarbonium ion (3) with a $^3H_4$-like half-chair conformation (Scheme 2). This conformation will energetically be rather unfavourable because of the four pseudo-axial substituents.

Alternatively, the $^4H_3$ conformation of the oxycarbonium species 3 is formed from the $B_{0,3}$ conformation of 1. The $^4H_3$ conformation lacks pseudo-axial interactions and will be more susceptible for reaction than the $^3H_4$ conformation.

In order to ascertain the most probable reaction pathways, the free enthalpies of the different reacting species have been estimated. The 3-amino-3-deoxy derivative of 1 has been shown to exist in solution as a mixture of the $^1C_4$ and $B_{0,3}$ conformation[19]. Although the $B_{0,3}$ conformation of 1 has not been observed in NMR experiments[20], MM-2 calculations suggest that the two conformations of 1 have similar energies[21]. On the other hand, the $^4H_3$ conformation of 3 is estimated to be 10 kJ/mol more favourable than its $^3H_4$ conformation using the method of Angyal[22]. Therefore, the reaction is thought to take place predominantly via the $B_{0,3}$ conformation as depicted in energetic terms in Fig. 6.

The ratio of attack by 1-butanol from the α- and β-side of 3 will be determined by steric and electronic factors, which are presently insufficiently understood for a useful prediction[23]. A high selectivity to α-D-glucosides from the $^4H_3$ conformation of 3, however, cannot be expected, in accordance with the experimental ratio of $k_1/k_2 = 1.5$.

The initial 2α/2β ratio from 1 (1.5) is somewhat higher than found starting from D-glucose (~ 1), but if the maximal yield of 2α is desired



Scheme 2. Possible mechanisms for the acid-catalysed butanolysis of 1 into 2α and 2β.

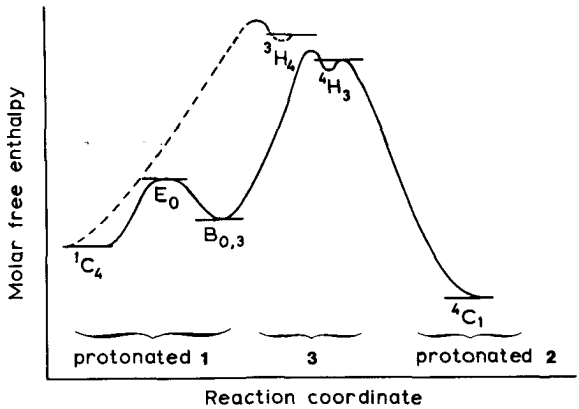


Fig. 6. Schematic representation of the relative free enthalpies for the possible pathways in the butanolysis of 1, assuming that the intermediate oxycarbonium ions 3 closely resemble the transition states of the reaction.

subsequent (slow) anomerisation to the thermodynamic ratio of 2α and 2β (2.7) is still required.

8.4 EXPERIMENTAL

Reaction procedure

1,6-Anhydroglucose[5] (m.p. 178-182°C) and butyl β-D-glucopyranoside[24] (m.p. 62°C) were > 99% pure according to HPLC.

Tubes were filled with a freshly prepared solution (~ 2 mL) of 1 or 2β (50.0 mM) and anhydrous[25] sulfuric acid (5.0 mM) in 1-butanol at 0°C. The tubes were sealed and heated in a water bath at 80.0°C. At regular intervals a tube was removed and cooled to 0°C. After opening 1.00 mL was pipetted off and neutralised with sodium hydrogen carbonate (1.00 mL, 10 mM). Mannitol was added as internal standard for HPLC analysis and 1-butanol was removed *in vacuo* at 50°C.

HPLC analysis

HPLC was performed on a M6000A system equipped with a R401 refractive index detector (Waters Assoc.). An Aminex HPX 87C column (300 x 7 mm, Bio-Rad) at 60°C was eluted with water at 0.6 mL/min[26]. Retention times

(min): 14.6, 2β; 17.8, 2α; 21.3, mannitol (internal standard); 25.3, 1. The response factor of 2α was supposed to be identical to that of 2β. All samples were analysed in duplicate. Concentrations have been expressed in mole fractions.

Linear regression

The data sets were fitted to the straight line y = p.x using the method of least squares, with calculation of the 95% confidence interval of the thus estimated value of p^{27}.

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CHAPTER 9

THE CRYSTAL STRUCTURE OF ANHYDROUS OCTYL α-D-GLUCOPYRANOSIDE. A COMPARISON WITH ITS HEMI- AND MONOHYDRATE*

9.1 INTRODUCTION

A new method for the preparation of alkyl α-D-glucopyranosides (alkyl = octyl, decyl, dodecyl) by selective crystallisation from a mixture with the β-anomer has recently been developed[1]. In order to achieve crystallisation in the presence of traces of water, further improvement of this method is under study[2]. Elucidation of the crystal structures of alkyl D-glucopyranosides might result in an optimisation of the crystallisation step, thus making octyl α-D-glucopyranoside much better available. Knowledge of the crystal structure will also be of importance to the study of alkyl α-D-glucopyranosides as liquid crystals[3] and membrane protein solubilising detergents[4].

Recently, we solved the crystal structure of octyl α-D-glucopyranoside monohydrate[5]. Simultaneously, Jeffrey, Yeon, and Abola[6] submitted a paper describing the identical structure. They also described a hemihydrate, which, like the monohydrate, consisted of alternating regions of polar and nonpolar groups, resulting from the close packing of fully extended hydrocarbon chains between hydrogen-bonded layers of glucopyranoside rings. In this paper the crystal structure of anhydrous octyl α-D-glucopyranoside is reported and compared to the hemihydrate and monohydrate crystals of the same compound and with the anhydrous decyl homologue[7].

* H. van Koningsveld, J.C. Jansen, and A.J.J. Straathof, Acta Crystallogr. Sect. C, in press.

9.2 EXPERIMENTAL

Octyl α-D-glucopyranoside monohydrate[1-2] was recrystallised from ethyl acetate. Anhydrous crystals were only obtained upon addition of zeolite KA, which adsorbed the dissolved water. An irregular-shaped crystal (maximum dimensions ~ 0.4 x 0.3 x 0.1 mm) was cut from a larger plate. Enraf-Nonius CAD-4 diffractometer, graphite monochromator, CuKα radiation. Cell dimensions from setting angles of 25 reflections with $23.5 \leq \theta \leq 39.5°$. 1760 unique reflections surveyed to $\theta_{max} = 76.25°$; h:0 → 6, k:0 → 9, l:-25 → 25; 1690 reflections with $I > \sigma(I)$. Three reference reflections monitored periodically showed no significant variation in intensity. No absorption correction. Structure determined by direct methods. H atoms located in a difference Fourier synthesis but included, except for the H atoms bonded to O, in idealized positions [d(C-H) = 0.96 Å]. Least-squares calculations on F with anisotropic thermal parameters for C and O atoms and fixed isotropic thermal parameters for C and O atoms and fixed isotropic values for H atoms. Absolute configuration chosen to be consistent with the configuration of the skeleton in previous analysis[6]. Convergence at R = 0.049, wR = 0.058, $w = 1/\sigma^2(F_o)$, S = 28.7 for 192 variable and 1747 observations [1682 with $I > \sigma(I)$ plus those for which $F_c > F_o$; 8 strong reflections left out final refinement cycles]; $(\Delta/\sigma)_{max} = 0.1$ [yH(20)]. Final ΔF synthesis has $\rho < 0.25$ eÅ^{-3}. All calculations performed on the Delft University Amdahl 470/V7B computer with programs of the *XRAY72*[8] and *MULTAN*[9] packages. Atomic scattering factors from *XRAY72*.

9.3 DISCUSSION

Fig. 1, drawn with *ORTEP*[10], shows the molecular conformation and atom labeling. Atomic coordinates are listed in Table 1 and molecular dimensions in Table 2. Besides the O(3)-H distance, no abnormal features in bond lengths and valence angles were found. From the ΔF map the (disordered?) position of the H atom bonded to O(3) could not be located accurately, resulting in a rather short O-H distance of 0.46 Å. The conformation is similar to those of the hemi- and monohydrate[6], and the anhydrous decyl compound[7]. The alkyl chain is fully extended and the pyranoside ring is slightly flattened with respect to the ideal chair conformation.

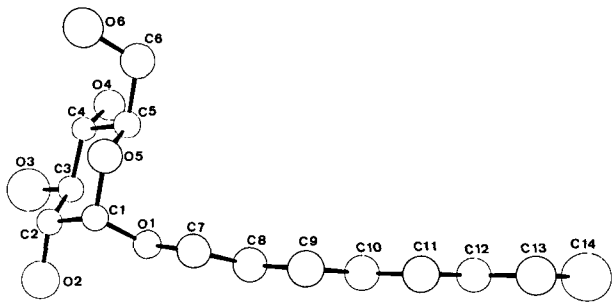

Fig. 1. Atomic notation and thermal ellipsoids, at 50% probability, for anhydrous octyl α-D-glucopyranoside.

Table 1. Atomic coordinates (x 10^4) and equivalent isotropic thermal parameters ($Å^2$ x 10^3). U_{eq} = 1/3 (U_{11} + U_{22} + U_{33}).

	x	y	z	U_{eq}
C(1)	1344(6)	2843(6)	1650(1)	29(1)
C(2)	1908(6)	2717(6)	906(1)	30(1)
C(3)	4286(6)	3822(6)	768(1)	31(1)
C(4)	3913(6)	5690(6)	1006(1)	28(1)
C(5)	3187(6)	5731(6)	1736(1)	31(1)
C(6)	2467(8)	7554(6)	1978(2)	39(1)
C(7)	2945(7)	1902(6)	2717(1)	37(1)
C(8)	5494(7)	1565(6)	3091(2)	39(1)
C(9)	5231(7)	1484(7)	3844(2)	40(1)
C(10)	7804(6)	1404(7)	4241(2)	39(1)
C(11)	7565(7)	1410(7)	4993(2)	40(1)
C(12)	10131(6)	1413(7)	5393(2)	39(1)
C(13)	9868(7)	1458(7)	6145(2)	43(1)
C(14)	12447(8)	1464(8)	6541(2)	55(2)
O(1)	3430(4)	2091(5)	2017(1)	32(1)
O(2)	2337(6)	946(5)	704(1)	44(1)
O(3)	4602(8)	3813[a]	59(1)	52(1)
O(4)	6282(5)	6641(5)	949(1)	37(1)
O(5)	934(5)	4627(5)	1833(1)	31(1)
O(6)	457(5)	8378(5)	1582(1)	43(1)

[a] Fixed parameter.

Table 2. Molecular geometry.

(a) Bond lengths (Å)

C(1)-C(2)	1.525(4)	C(5)-O(5)	1.449(5)
C(2)-C(3)	1.517(5)	C(6)-O(6)	1.422(5)
C(3)-C(4)	1.512(6)	C(7)-O(1)	1.434(4)
C(4)-C(5)	1.518(4)	C(7)-C(8)	1.504(5)
C(5)-C(6)	1.518(6)	C(8)-C(9)	1.515(4)
C(1)-O(1)	1.398(4)	C(9)-C(10)	1.515(5)
C(1)-O(5)	1.422(6)	C(10)-C(11)	1.510(4)
C(2)-O(2)	1.425(6)	C(11)-C(12)	1.515(5)
C(3)-O(3)	1.428(4)	C(12)-C(13)	1.512(5)
C(4)-O(4)	1.425(5)	C(13)-C(14)	1.516(5)

(b) Bond angles (°)

O(1)-C(1)-C(2)	108.3(3)	C(4)-C(5)-C(6)	113.3(3)
O(1)-C(1)-O(5)	111.9(3)	C(4)-C(5)-O(5)	110.0(3)
O(5)-C(1)-C(2)	110.2(3)	O(5)-C(5)-C(6)	106.3(3)
C(1)-C(2)-C(3)	109.1(3)	C(5)-C(6)-O(6)	114.0(3)
C(1)-C(2)-O(2)	111.7(3)	C(1)-O(5)-C(5)	113.1(3)
O(2)-C(2)-C(3)	109.7(3)	C(1)-O(1)-C(7)	113.1(2)
C(2)-C(3)-C(4)	110.5(3)	O(1)-C(7)-C(8)	108.5(3)
C(2)-C(3)-O(3)	107.5(3)	C(7)-C(8)-C(9)	112.9(3)
O(3)-C(3)-C(4)	109.6(3)	C(8)-C(9)-C(10)	114.2(3)
C(3)-C(4)-C(5)	111.1(3)	C(9)-C(10)-C(11)	114.6(3)
C(3)-C(4)-O(4)	109.5(3)	C(10)-C(11)-C(12)	114.9(3)
O(4)-C(4)-C(5)	108.0(3)	C(11)-C(12)-C(13)	114.4(3)
		C(12)-C(13)-C(14)	114.0(3)

(c) Selected torsion angles (°) (e.s.d.'s ~ 0.4°)

O(5)-C(1)-C(2)-C(3)	58.3	O(1)-C(7)-C(8)-C(9)	4.4
C(1)-C(2)-C(3)-C(4)	54.9	C(7)-C(8)-C(9)-C(10)	8.2
C(2)-C(3)-C(4)-C(5)	53.6	C(8)-C(9)-C(10)-C(11)	2.8
C(3)-C(4)-C(5)-O(5)	54.1	C(9)-C(10)-C(11)-C(12)	2.6
C(4)-C(5)-O(5)-C(1)	59.1	C(10)-C(11)-C(12)-C(13)	1.2
C(5)-O(5)-C(1)-C(2)	61.7	C(11)-C(12)-C(13)-C(14)	0.1

Table 2 (continued)

(d) Hydrogen-bonding scheme

X-H---Y	X---Y	X-H	H---Y	X-H---Y
O(2)-H(20)---O(6^{i})	2.82(5)	0.78(5)	2.05(5)	166(5)
O(3)-H(30)---O(2^{ii})	2.76(4)	0.46(7)	2.34(7)	153(10)
O(4)-H(40)---O(3^{ii})	2.63(4)	0.81(4)	1.85(5)	161(5)
O(6)-H(60)---O(4^{iii})	2.78(4)	0.86(5)	2.00(4)	151(4)

Symmetry code: (i) x, y-1, z; (ii) -x + 1, y + $^{1}/_{2}$, -z; (iii) x + 1, y, z.

The molecular packing is illustrated in Fig. 2A. The structure consists of alternating regions of polar and nonpolar groups, and is strictly analogous to that of the decyl compound. The lengthening of the *c*-axis in the decyl compound with respect to the octyl compound is caused by the longer alkyl chain. The other cell dimensions are hardly affected (Table 3).

The cell dimensions of anhydrous octyl α-D-glucopyranoside (Z = 2) show a remarkable correspondence to those of the hemihydrate (Z = 4): *a* of the anhydrous crystal is equal to *b* of the hemihydrate, *b* of the anhydrous crystal is equal to 0.5 *a* of the hemihydrate, and the *c* axes are the same in both crystals. This indicates an equivalent total void volume per octyl α-D-glucopyranoside molecule (Table 3). The anhydrous crystal (Fig. 2A) can be transformed to the hemihydrate crystal (Fig. 2B) by a rotation of 180° around an axis parallel to *c* of one of the carbohydrate molecules in the anhydrous unit cell. In doing so, the system of voids of equivalent size between glucopyranoside layers in the anhydrous compound (hatched in Fig. 2A) is transformed into a system of alternating smaller and larger voids (hatched in Fig. 2B) without having any effect on the total void volume. The original voids as well as the smaller voids formed upon transformation are incapable of occluding water molecules. Upon incorporation of a water molecule in the larger voids, the hemihydrate structure is obtained, having infinite chains of hydrogen bonds in an order O-H(20) --- O-H(60) --- O-H(40) --- O-H(30) --- O-H(w) --- O-H(20) ---. A similar scheme, without O-H(w) ---, is present in the anhydrous crystal (*cf.* Table 2d and ref. 6).

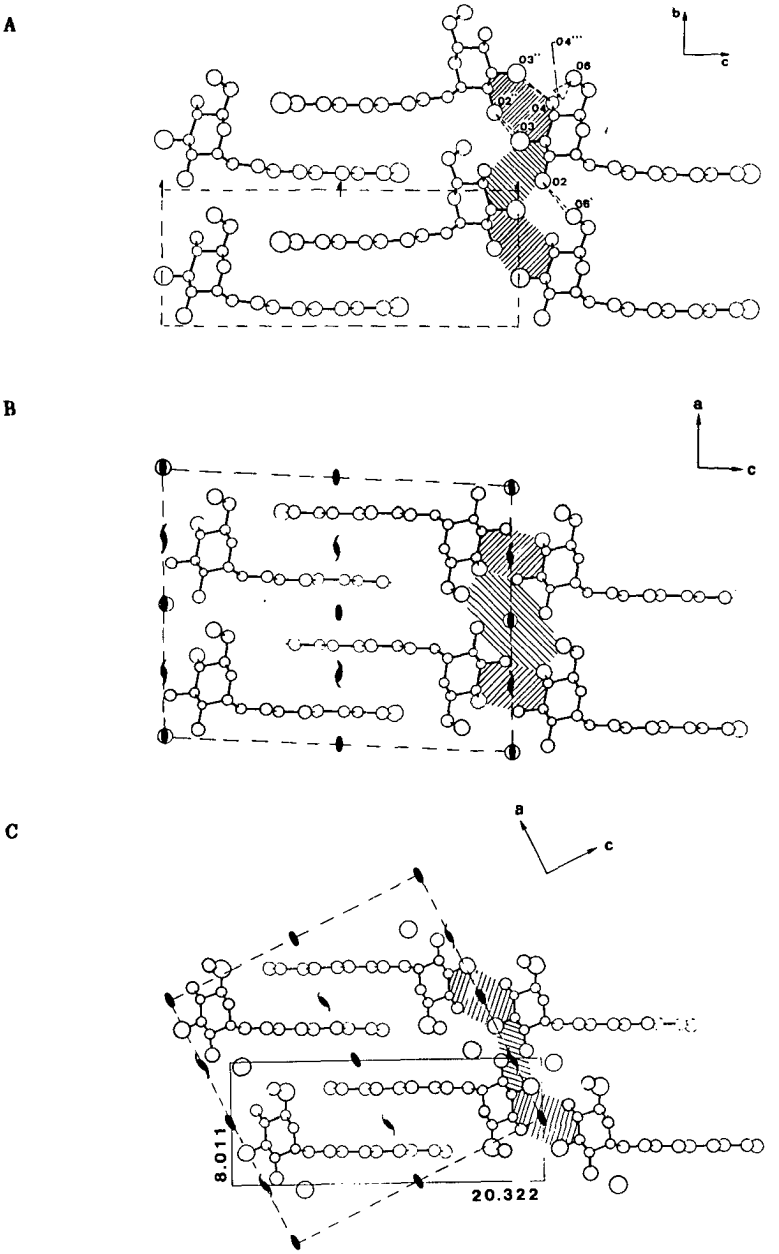

Fig. 2. Molecular packing in the crystal structures of octyl α-D-glucopyranoside (A) *in the anhydrous crystal,* (B) *in the hemihydrate crystal (ref. 6),* (C) *in the monohydrate crystal (subcell indicated by full lines). In the three structures, corresponding voids are dotted.*

Table 3. Comparison of anhydrous octyl α-D-glucopyranoside with the hemihydrate and the monohydrate, and with anhydrous decyl α-D-glucopyranoside.

	Spgr	*a* (Å)	*b* (Å)	*c* (Å)	β (°)	Z	V/Z (Å^3)
octyl							
$C_{14}H_{28}O_6$ [a]	$P2_1$	5.140(2)	7.604(2)	19.939(4)	92.18(2)	2	389.4
$C_{14}H_{28}O_6 \cdot {}^1/_2\ H_2O$ [b]	$C2$	15.190(5)	5.136(3)	19.944(7)	92.74(3)	4	388.6
$C_{14}H_{28}O_6 \cdot\ 1\ H_2O$ [c]	$C2$	17.829(3) (8.011)	5.144(2) (5.144)	18.262(4) (20.322)	90.30(2)	4	418.7
decyl							
$C_{16}H_{32}O_6$ [d]	$P2_1$	5.153(2)	7.624(4)	22.125(7)	90.95(4)	2	434.7

[a] This work. [b] Ref. 6. [c] Ref. 5. Within parentheses the subcell values are given. [d] Ref. 7.

In the monohydrate a subcell very similar to the unit cell in the anhydrous and hemihydrate crystals can be outlined (see Fig. 2 and Table 3). The main difference with the hemihydrate structure can be described as a shift of neighbouring (201)-layers, 8.011 Å thick. The hydrogen bonding scheme in the monohydrate crystal is therefore different from those in the hemihydrate and anhydrous crystals.

The alkyl chain packing seems to be similar in Figs. 2A, B, and C. However, in the anhydrous crystal the 2_1-axis is perpendicular to the alkyl chain plain whereas in the hemihydrate and monohydrate structures the 2_1-axis is parallel to the alkyl plane. Thus, all chains are parallel in the anhydrous crystal seen along *b*. The interwoven chains cross each other in both the hemihydrate and monohydrate crystals seen along *a*.

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CHAPTER 10

SOLID STATE AND SOLUTION PROPERTIES OF OCTYL D-GLUCOPYRANOSIDES

10.1 INTRODUCTION

Recently we have developed an efficient procedure for the one-step preparation of octyl α-D-glucopyranoside (1α) from D-glucose and 1-octanol[1]. Crystalline 1α monohydrate was easily obtained upon addition of petroleum ether (b.p. 40-65°C) to the reaction mixture. The β-isomer (1β) remained in solution, together with various octyl oligoglucosides, and was recycled for subsequent conversion into 1α.

By contrast, pure 1β is usually prepared by a selective but three-step synthesis according to the Koenigs-Knorr procedure[2-4]. Because of its excellent protein solubilising ability 1β is being used extensively in biological membrane research[4-5]. The high critical micelle concentration (CMC) of 1β (26-23 mM in water at 20-30°C[6-7]) seems to be advantageous in this respect. The micellisation process can be considered to be a rapid equilibrium between monomeric molecules of 1β and a micellar associate of 1β[8]. At 20°C, the aggregation number N has been estimated to be 68.3 and the association constant $K = [N\text{-mer}]/[1\beta]^N$ to be 10^{99}[8]. A duplex micelle, consisting of a reverse micelle within a normal micelle, has been proposed[9].

Because of a Krafft-point at ~ 40°C, 1α seems to be less useful than its β-anomer for membrane protein solubilisation[5]. The remarkable differences in properties of 1α and 1β, some of which have been described by Dorset and Rosenbusch[10], induced us to a further study, the more so because recently we[11] as well as Jeffrey *et al.*[12] observed the occurrence of several crystalline forms of 1α which had not been recognised previously.

10.2 RESULTS AND DISCUSSION

At 25°C, the 1-octanol to water partition coefficient (P) and the solubility (S) in 1-octanol are comparable for 1α and 1β (Table 1). The

fairly high P and S values probably originate from mixed reverse micelle formation of 1α and 1β with 1-octanol. Large differences in solubility are observed in diethyl ether (~ 1 g/L for 1α and > 7 g/L for 1β at 20°C) as well as in water. Between 0 and 50°C the aqueous solubility of 1β exceeds 100 g/L, whereas Fig. 1 shows that at 20°C only 6 g/L 1α dissolves. Aqueous solutions of 1α, however, show a Krafft-point at 40°C, *i.e.* the temperature at which the concentration of dissolved (monomeric) 1α has become large enough for micellisation to occur[13]. Because of this Krafft-point recrystallisation of 1α from water is easily performed.

Table 1. 1-Octanol-water partition coefficients (P) *and solubilities* (S) *in 1-octanol of 1α and 1β.*

Anomer	Temperature (°C)	P [a]	S (g/L)
α	25	34	23
α	35	36	55
α	45	49	170
β	25	29	30

[a] 100 mg 1, 5 mL 1-octanol, 5 mL water.

Brown *et al.*[14] interpreted a discontinuity of the density *vs.* concentration curve of aqueous 1α at 25°C as a CMC value (10 mM; 3g/L), but our data indicate that these authors in fact measured the solubility limit. A true CMC (9.4 mM) is obtained from surface tension measurements at 42°C, *i.e.* above the Krafft-point (Fig. 2). At this temperature, the CMC of 1β is found to be 21 mM, thus ΔΔG for micellisation[15] between 1α and 1β is equal to RT ln ΔCMC = -2.1 kJ/mol. This difference, as well as the decrease of CMC of 1β with increasing temperature (Fig. 1), will be the result of the contribution of different enthalpy and entropy changes of both hydrophilic and hydrophobic groups upon micellisation[16].

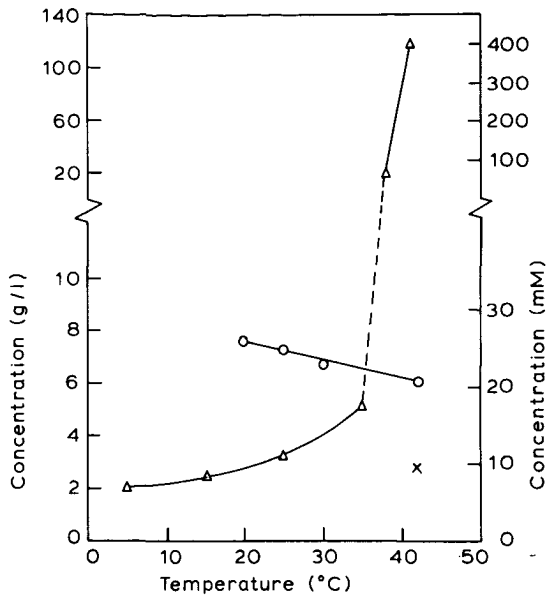

Fig. 1. Aqueous solubility (Δ) *and CMC* (×) *of octyl α-D-glucopyranoside; CMC of octyl β-D-glucopyranoside* (o, *values for 20 and 30°C from ref. 6 and for 25°C from ref. 7).*

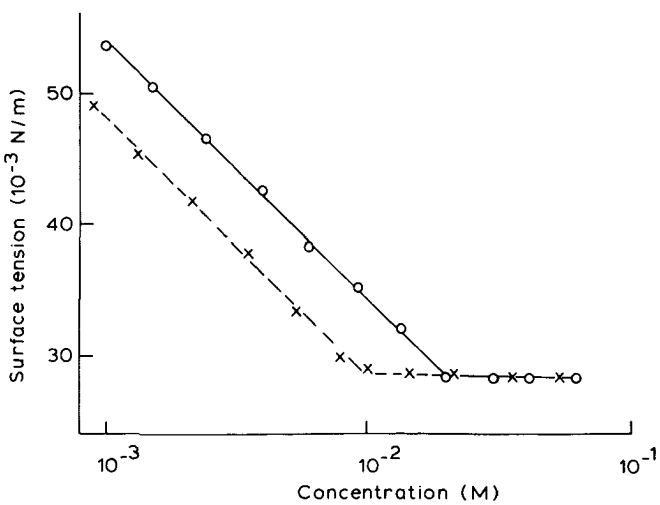

Fig. 2. Surface tension of aqueous octyl α-D-glucopyranoside (×) *and β-D-glucopyranoside* (o) *as a function of the concentration (42°C).*

Since micellisation occurs preferably for 1α, it cannot account for the larger aqueous solubility of 1β. A better hydration of monomeric 1β is also improbable as an explanation. The solvation of methyl α- and β-D-glucopyranoside in water do not seem to differ significantly according to their almost equal hydration number[17] and apparent molal volume[18]. For 1α and 1β back-folding of the octyl chain to the non-polar underside of the D-glucose ring might occur in addition, but this would induce less torsional strain on the octyl chain for 1α than for 1β and would increase the relative solubility of 1α.

The data point to a much better crystal packing for 1α than for 1β as the only significant origin of the solubility differences. In the anhydrous crystal of 1α[11], as well as in the hemihydrate and monohydrate crystal[12],

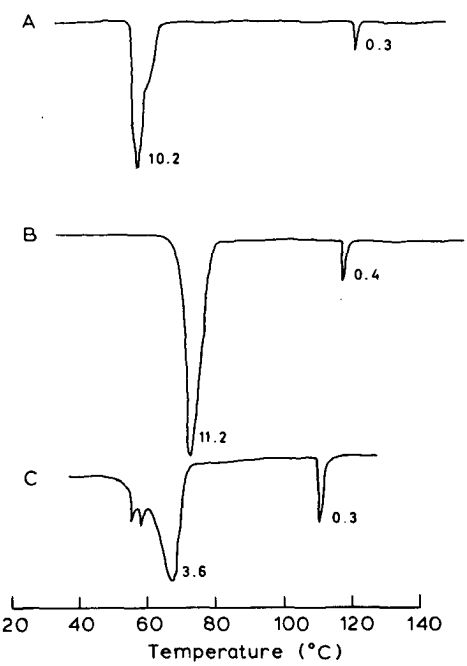

Fig. 3. DSC of anhydrous octyl α-D-glucopyranoside (A), *octyl α-D-glucopyranoside monohydrate* (B), *and octyl β-D-glucopyranoside* (C). *Heating rate 5°C/min. Numbers indicate endothermic transition enthalpies in MJ/mol.*

the maximum number of infinite -chains of hydrogen bonds is present. In addition, the octyl chain packing is very favourable for all these types of crystal. No hydrated crystals of 1β are known, and so far no X-ray structure of anhydrous 1β has been solved, but DSC reveals that the crystal packing of 1β will be much less ideal than for 1α. Crystals of 1α or 1β both exhibit phase transitions to anisotropic liquids (liquid crystals) upon heating before they melt to an isotropic liquid[19]. The enthalpy required for melting 1β is less than half of that for 1α or 1α monohydrate (Fig. 3), which explains the solubility differences.

In analogy, in crystals of *N*-nonanoyl-*N*-methyl-D-glucamine hydrogen-bonding was rather weak, and the compound was easily soluble in water[20]. In contrast, the closely related surfactant *N*-octyl-D-gluconamide showed a Krafft-point at 90°C because of much stronger hydrogen-bonding[21] in the crystal.

It should be noted that, for octyl D-glucosides, data from DSC or XRD should be interpreted carefully, since crystal to crystal transition of 1α upon hydration occurred in some instances. Considering the wide scatter in melting and clearing points reported for 1β (*cf. e.g.* refs. 2, 3, 8, and 10) different crystalline forms of this compound might exist as well.

10.3 EXPERIMENTAL

Anhydrous octyl α-D-glucopyranoside[1] crystals were obtained from ethyl acetate in the presence of zeolite KA. The monohydrate of 1α was crystallised from water. Octyl β-D-glucopyranoside (anhydrous according to elemental analysis) was obtained from Sigma. HPLC determination of 1 was performed on an Aminex HPX 87C column at 60°C, which was eluted with 0.01 M aqueous trifluoroacetic acid[1]. Surface tensions were determined with a Du Nouy type tensiometer at 42°C. For DSC, the samples were encapsulated in hermetic sealing pans, made of aluminium. Experiments were performed on a Du Pont 910 DSC apparatus at a heating rate of 5°C/min[22]. Solubilities were determined by stirring thermostatted suspensions of 1α or 1β for > 1 h, settling for > 1 h, and HPLC analysis of the clear solution. Partition coefficients were determined by stirring a mixture of 100 mg 1, 5 mL water, and 5 mL 1-octanol and HPLC analysis of both layers after settling.

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SUMMARY

This thesis deals with alkyl glycoside surfactants from starch and sucrose.

Chapter 1 reviews the preparation and application of surfactants, and the present status of carbohydrate-derived surfactants in this respect. The limiting conditions for the preparation of potential surfactants from carbohydrates by new routes or new methods are evaluated.

Chapter 2 and 3 deal with the scope and limitations of the application of the enzyme invertase to the preparation of alkyl fructosides. Invertase transfers the fructosyl group of sucrose to either primary alcohols or water. In concentrated solutions of sucrose (Chapter 2), fructosyl transfer to sucrose, glucose, and fructose occurs, leading to the formation of oligosaccharides such as 6-kestose. The selectivity of oligosaccharide formation relative to sucrose hydrolysis is found to decrease with increasing concentration of sucrose. In mixtures of water and primary alcohols (Chapter 3), invertase converts sucrose into alkyl β-D-fructofuranosides. Under anhydrous conditions, no fructoside formation is observed, but the kinetics of the competing reactions of sucrose with water and primary alcohol as fructosyl acceptor show that on a molar basis water is a relatively unreactive acceptor. The limitations of this enzymic method for alkyl fructoside formation are explained by assuming a nonspecific non-polar binding site to be present in the enzymic cavity near the specific polar binding site for the β-D-fructofuranosyl group of the substrate.

Chapter 4 deals with the esterification of the bis(tetrabutylammonium) salt of α-D-glucopyranosyl phosphate. With benzyl chloride or bromide 80-90% α-D-glucopyranosyl benzyl phosphate is obtained. An excess of benzyl chloride catalyses further reaction to a mixture of six phosphate diesters via phosphate triesters and glucopyranosyl chlorides as intermediates. 1-Bromohexane gives α-D-glucopyranosyl hexyl phosphate by the same method.

In Chapter 5 the course of the reaction of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose in 1-butanol is described. Using ion-exchange resin ($-SO_3H$) as the acidic catalyst up to 90% butyl D-glucoside is formed. In 1-

octanol, a comparable reaction mixture is obtained, from which octyl α-D-glucopyranoside is crystallised in 30% yield. 1-Decanol and 1-dodecanol give crystalline α-D-glucopyranosides by the same method.

Chapter 6 deals with the conversion of glucose in 1-octanol using various macroporous sulfonic acid ion-exchange resins as the catalyst. This reaction results in an equilibrium mixture consisting of octyl D-glucosides together with substantial amounts of reversion products. Octyl α-D-glucopyranoside monohydrate selectively crystallises from the reaction mixture upon addition of petroleum ether (b.p. 40-65°C). The mother liquor is recirculated to the reactor and re-equilibrated in the presence of additional glucose and 1-octanol. The ion-exchange resin and petroleum ether are also recycled. This procedure allows the one-step preparation of crystalline octyl α-D-glucopyranoside monohydrate in a quantitative yield from glucose and 1-octanol without the use of toxic solvents or the formation of waste products.

Chapter 7 deals with the application of micowave technology to the preparation of 1,6-anhydroglucose. Thermolysis of starch or other (1→4)-glucans yields this compound within 10 min in a convential microwave oven. The yield is not correlated to the molecular structure, degree of polymerisation, or water content of the substrate. A higher density of the substrate, however, minimises heat losses to the surroundings and gives better yields. The method is applicable to the simple and rapid preparation of < 1 g 1,6-anhydroglucose from starch.

In Chapter 8 the sulfuric acid-catalysed reaction of 1,6-anydroglucose in 1-butanol is described. Under anhydrous conditions butyl α- and β-D-glucopyranosides are the sole products. Their initial ratio is 1.5 but they equilibrate to the ratio 2.7. The magnitude of the four pseudo first order reaction rate constants involved have been determined. Conformational studies show that the reaction will probably proceed via a $^{1}C_{4}$-$B_{0,3}$ interconversion of 1,6-anhydroglucose and a $^{4}H_{3}$-like oxycarbonium ion species.

Chapter 9 deals with the crystal structure of anhydrous octyl α-D-glucopyranoside. The structure consists of alternating regions of polar and nonpolar groups resulting from the close packing of fully extended hydrocarbon chains between hydrogen-bonded layers of glucopyranoside rings. The hydrogen bonding scheme greatly resembles the hydrogen bonding in octyl α-D-glucopyranoside hemihydrate, but deviates from that in the monohydrate crystal. The octyl chain packing is different from the chain packing in both the hemihydrate and monohydrate crystals.

Finally, in Chapter 10 some solution and solid state properties of octyl α- and β-D-glucopyranoside are compared. The β-anomer shows a much better solubility in water, although the α-anomer has a larger tendency for micelle formation. Comparison of the phase transition to the liquid crystal indicates that the β-anomer will have a crystal packing which is much less ideal than those observed for the α-anomer.

SAMENVATTING

Dit proefschrift behandelt oppervlakte-actieve alkylglycosiden die van zetmeel en sucrose afleidbaar zijn.

Hoofdstuk 1 geeft een overzicht van de bereiding en toepassing van oppervlakte-actieve verbindingen, en de stand van zaken op het gebied van deze verbindingen in zoverre ze van koolhydraten afgeleid zijn. De mogelijkheden en beperkingen voor de bereiding ervan langs nieuwe routes worden geëvalueerd.

Hoofdstuk 2 en 3 behandelen de toepasbaarheid van het enzym invertase voor de bereiding van alkylfructosiden. Invertase draagt de fructosylgroep van sucrose over op zowel primaire alcoholen als op water. In geconcentreerde oplossingen van sucrose (Hoofdstuk 2) vindt fructosyl-overdracht plaats op sucrose, glucose en fructose, wat leidt tot de vorming van oligosacchariden zoals 6-kestose. In mengsels van water en primaire alcoholen (Hoofdstuk 3) zet invertase sucrose om in alkyl-β-D-fructofuranosiden. Onder watervrije condities wordt geen vorming van fructoside waargenomen, maar de kinetiek van de concurrerende reacties van sucrose met water en met primaire alcohol als fructosyl acceptor toont aan dat, op molaire basis, water een relatief weinig reactieve acceptor is. De beperkingen van deze enzymatische methode voor alkylfructoside-vorming worden verklaard met de aanname dat er zich een niet-specifieke apolaire bindingspositie in de enzymholte bevindt naast de specifieke polaire bindingspositie voor de β-D-fructofuranosylgroep van het substraat.

Hoofdstuk 4 behandelt de verestering van het bis(tetrabutylammonium)-zout van α-D-glucopyranosylfosfaat. Met benzylchloride of -bromide wordt 80-90% α-D-glucopyranosylbenzylfosfaat verkregen. Een overmaat benzylchloride katalyseert verdere reacties tot een mengsel van zes fosfaatdiesters via fosfaattriesters en glucopyranosylchlorides als tussenproducten. Met 1-broomhexaan wordt α-D-glucopyranosylhexylfosfaat verkregen volgens dezelfde methode.

In Hoofdstuk 5 wordt het verloop van de reactie van 1,2:5,6-di-*O*-isopropylideen-α-D-glucofuranose in 1-butanol beschreven. Met ionenwisselaarshars ($-SO_3H$) als de zure katalysator wordt butyl-D-glucoside ($\leq$ 90%) gevormd. In

1-octanol wordt een vergelijkbaar reactiemengsel verkregen, waaruit octyl-α-D-glucopyranoside kristalliseert in een opbrengst van 30%. 1-Decanol en 1-dodecanol geven kristallijne α-D-glucopyranosiden volgens dezelfde methode.

Hoofdstuk 6 behandelt de omzetting van glucose in 1-octanol met verschillende macroporeuze sulfonzure ionenwisselaarsharsen als katalysator. Deze reactie resulteert in een evenwichtsmengsel dat bestaat uit octyl-D-glucosiden en een aanzienlijke hoeveelheid reversieproducten. Octyl-α-D-glucopyranoside monohydraat kristalliseert selectief uit dit mengsel na toevoeging van petroleumether (kpnt. 40-65°C). De moederloog wordt gerecirculeerd naar de reactor en opnieuw omgezet tot een evenwichtsmengsel na aanvulling met glucose en 1-octanol. De ionenwisselaarshars en petroleumether worden eveneens gerecirculeerd. Deze procedure maakt het mogelijk om in één stap kristallijn octyl-α-D-glucopyranoside monohydraat te bereiden in een kwantitatieve opbrengst vanuit glucose en 1-octanol zonder bijproducten te verkrijgen of toxische oplosmiddelen te gebruiken.

Hoofdstuk 7 behandelt de toepassing van microgolftechnologie op de bereiding van 1,6-anhydroglucose. Thermolyse van zetmeel of andere (1→4)-glucanen leidt in een conventionele magnetronoven binnen 10 minuten tot de vorming van deze verbinding. De opbrengst is niet gecorreleerd met de moleculaire structuur, de polymerisatiegraad of het watergehalte van het substraat. Daarentegen vermindert een hogere dichtheid van het substraat warmteverliezen aan de omgeving, wat tot betere opbrengsten leidt. De methode kan gebruikt worden om snel en eenvoudig < 1 g 1,6-anhydroglucose uit zetmeel te bereiden.

In Hoofdstuk 8 wordt de zwavelzuur-gekatalyseerde reactie van 1,6-anhydroglucose in 1-butanol beschreven. Onder watervrije condities zijn butyl-α- en -β-D-glucopyranoside de enige producten. Hun verhouding, die aanvankelijk 1,5 is, stijgt tot 2,7 terwijl zich een evenwicht instelt. De grootte van de vier pseudo-eerste-orde reactiesnelheidsconstanten die een rol spelen is bepaald. Conformatie-analyse toont aan dat de reactie waarschijnlijk via een $^{1}C_{4}$-$B_{0,3}$ overgang van 1,6-anhydroglucose en een $^{4}H_{3}$-gelijkend oxycarbonium-ion verloopt.

Hoofdstuk 9 behandelt de kristalstructuur van watervrij octyl-α-D-glucopyranoside. De structuur bevat alternerende gebieden met polaire en apolaire groepen, die het gevolg zijn van de dichte pakking van volledig gestrekte koolwaterstofketens tussen waterstof-gebonden lagen van glucopyranoside ringen. Het schema van waterstofbindingen vertoont grote overeenkomsten met dat in octyl-α-D-glucopyranoside hemihydraat, maar wijkt af van dat in het

kristal van het monohydraat. De octyl-ketenpakking verschilt van de ketenpakking in het kristal van zowel het hemihydraat als het monohydraat.

Tenslotte worden in Hoofdstuk 10 enkele eigenschappen van octyl-α- en -β-D-glucopyranoside in oplossing en in de vaste stof vergeleken. Het β-anomeer vertoont een veel betere oplosbaarheid in water, hoewel het α-anomeer een grotere neiging tot micelvorming heeft. Vergelijking van de overgang naar het vloeibaar kristal toont aan dat het β-anomeer een kristalpakking moet hebben die veel minder ideaal is dan de pakking die voor het α-anomeer bekend is.

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CURRICULUM VITAE

Adrianus (Adrie) Johannes Jozef Straathof werd op 25 februari 1960 geboren in Den Haag. Na het behalen van het diploma Gymnasium β op het Edith Stein College te Den Haag begon hij in 1978 met de studie Scheikundige Technologie aan de Technische Hogeschool te Delft. Het ingenieursexamen werd in januari 1984 afgelegd.

In aansluiting op zijn afstudeerwerk begon hij in februari 1984 een promotieonderzoek onder leiding van prof. dr. ir. H. van Bekkum en dr. ir. A.P.G. Kieboom op het Laboratorium voor Organische Chemie. Na 50 maanden, inmiddels aan de Technische *Universiteit* Delft en met *prof.* Kieboom als eerste promotor, heeft dit geresulteerd in het proefschrift dat thans voor u ligt.